VOL. X, No. 3 MARCH 1959

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

UNIVERSITY OF HAWAII LIBRARY
JUN 8 '59

MARTINUS NIJHOFF — PUBLISHER — THE HAGUE

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol

Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten

Issued under the auspices of the
"Koninklijk Genootschap voor Landbouwwetenschap"
(Royal Netherlands Society of Agricultural Science)

Executive Editors: D. I. Arnon, Berkeley; J. Baeyens, Louvain; F. C. Gerretsen, Groningen; G. W. Harmsen, Groningen; E. G. Mulder, Wageningen; F. J. Richards, London; A. C. Schuffelen, Wageningen; F. Steenbjerg, Copenhagen. **Secretary:** G. H. Arnold, Groningen.

Consulting Editors: Wm. A. Albrecht, Columbia, Mo.; C. Arnaudi, Milan; E. Gäumann, Zurich; L. Gisiger, Liebefeld-Berne; F. G. Gregory, London; A. J. de Groot, Groningen; J. den Holder, Ghent; T. H. van den Honert, Leyden; V. J. Koningsberger, Utrecht; J. Lavollay, Paris; W. J. Lütjeharms, Bloemfontein; H. Lundegårdh, Uppsala; E. Melin, Uppsala; M. Mes, Pretoria; M. Ødelien, Ås; E. J. Petersen, Copenhagen; C. S. Piper, Adelaide; H. G. Thornton, Harpenden, Herts.; S. Tovborg Jensen, Copenhagen; A. I. Virtanen, Helsinki; S. A. Waksman, New Brunswick; F. W. Went, Pasadena; Joh^{a}. Westerdijk, Baarn; J. G. Wood, Adelaide.

CONTENTS: TABLE DES MATIÈRES: INHALT:

Somir Banerjee, Some studies of salt-affected soils of Canning, West Bengal 207

Y. T. Tchan, Study of soil algae. III. Bioassay of soil fertility by algae . . 220

James A. Goss and *E. M. Romney*, Effects of bicarbonate and some other anions on the shoot content of P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, Sr^{90}, Ru^{106}, Cs^{137}, and Ce^{144} in bean and barley plants 233

Roderick P. Murphy, Some factors influencing cation uptake by excised roots of perennial rye grass *(Lolium perenne)* 242

Chresten Sorensen, The influence of nutrition on the nitrogenous constituents of plants I. Nitrogen and phosphorus pot experiments with oats 250

S. K. Majumder and *Stuart Dunn*, Use of organic phosphate in nutrient culture 266

J. D. Ovington and *H. A. I. Madgwick*, The growth and composition of natural stands of birch. I. Dry-matter production 271

F. Steenbjerg and *Sv. T. Jakobsen*, Some approaches to experimental investigations into the correlation between the slope and the sigmoidal shape of yield curves 284

Letter to the editors. *S. K. Majumder* and *Stuart Dunn*, Modifying effect of EDTA on the copper toxicity to corn in nutrient solution 296

Letter to the editors. *K. H. Schütte* and *P. Martin*, The influence of certain micro-nutrient deficiencies on the germination rates of radish and millet . . 299

Printed in the Netherlands

Plant and Soil X. 207—302 The Hague March 1959

Plant and Soil X, no. 3 March 1959

SOME STUDIES OF SALT-AFFECTED SOILS OF CANNING, WEST BENGAL

by SOMIR BANERJEE

State Agricultural Research Institute, Calcutta-40, India

Land available in India for agricultural purposes is very much limited in proportion to the population. This is especially true in the case of West Bengal, one of the provinces in the eastern part of India.

There is a considerable area in the southern part of Gangetic West Bengal comprising the estuarine tracts on the seaboard side where inadequate drainage and salty conditions limit soil productivity. Though some data on the appraisal of soil salinity in the saline and alkali soils of India which occur in the arid and semi-arid regions are available, very little work has been done on the saline and alkali soils of the humid, estuarine areas of India and other tropical countries. Underlying principles relating to soil properties and plant responses apply equally well to both cases. The main difference is that in humid climates precipitation exceeds consumption so that if drainage is adequate, the excess soluble salts may be leached out of the soil by rainwater. Economically, in humid climates, the most important consideration of soil salinity and exchangeable sodium has been in connection with the drainage and reclamation of soils underlying salty lakes and shallow coastal waters.

The reclamation of soils that have been subjected to sea-water inundation is an agricultural problem that has been given a great deal of attention by soil and plant scientists. This problem is particularly serious when it occurs on older cultivated soils in humid regions, because there is a lack of soluble calcium for replacing exchangeable sodium concurrently with the leaching out of the soluble salts. Leaching by rainwater changes the soil from the saline alkali to the non-saline alkali condition, with the attendant deterio-

ration of soil structure. Reclamation then requires the application of soluble calcium to replace the exchangeable sodium together with a period of careful cultural and management practices to re-establish a favourable physical status of the soil. Van den Berg [10] provides an introduction to the literature on this subject.

The reclamation of salt-affected soils, in spite of the initial difficulties that may occur, is a problem, which if tackled properly can bring immense benefit to a country. The pioneer work done in this respect in the Netherlands has established beyond doubt how some of the complex problems preceding and following reclamation can be successfully tackled. Zuur [11] has sketched historical and technical aspects and has given an introduction to the extensive literature of the Netherlands in this subject. Although conditions existing in the Netherlands are not identical with conditions prevalent elsewhere, sufficient similarities, nevertheless, do exist to render the task of reclamation elsewhere somewhat easier. It is especially valuable that some of the techniques and methods commonly employed for reclamation in the Netherlands have been thoroughly tested and developed to a high degree of perfection. It might be added in this connection that soil conditions found in the estuarine tracts of the Gangetic delta of West Bengal typify soil conditions which may be presumed to occur in other low countries of the humid tropics, though the magnitude of the problem may vary from one place to another.

However, before any attempt at reclamation is tried in these salt-affected soils of Gangetic West Bengal, it is imperative that the salinity status of these soils be determined. This is all the more important since very little of such work has been done and analytical data in respect of these saline and alkali soils are extremely meagre. In this investigation, an attempt has been made to appraise the soil conditions in twelve mauzas covering an area of approximately three hundred square miles which fall under the jurisdiction of police station Canning. The aim was to devise methods for increasing the productivity of the land. The hydrological and drainage conditions, and other relevant features of this area are more or less representative of the conditions prevalent in the neighbouring estuarine areas. It goes without saying, however, that the limited number of samples studied permits very restricted conclusions and at the same time gives very preliminary indications.

DESCRIPTION OF THE AREA

The area referred to above falls within the seaboard face of the Gangetic delta of West Bengal, and its physical features are those

common to deltaic, estuarine lands. The land is flat, it is little raised above flood level and the highest ground is that bordering the river channels. As one approaches the coast, the land gradually declines to an elevation which throughout many hundred square miles is scarcely raised above the high-water mark. This seaboard area is a typical specimen of new deltaic formation – it exhibits the process of land formation in an unfinished state.

The average annual rainfall is about 175 cm, eighty to ninety per cent of which falls during the monsoon months of June through October.

MATERIALS AND METHODS

Soils from six profiles were chemically examined and analysed in the laboratory. Mechanical analysis of soils was carried out according to Robinson's pipette method. pH and the total soluble-salt content (expressed as per cent of air-dried soil) were determined using a soil:water ratio of 1 : 5 by means of a Philips pH meter (employing a glass electrode) and a Philips conductivity bridge apparatus respectively. The hydrochloric acid extracts were analysed following the methods given in Piper's "Soil and Plant Analysis" [7], with the exception that potassium was determined by means of a Lange flame photometer. Organic carbon was determined by Walkley and Black's rapid titration method, while nitrogen was determined by Bal's modified Kjeldahl method. Total cation-exchange capacity was determined by saturating the soil with barium chloride, extracting and then estimating the barium by precipitating as barium sulphate. Since the soils contained little or no carbonates, this method was expected to give satisfactory results. Sodium and potassium were determined by means of a Lange flame photometer, while calcium and magnesium in the water extracts were determined by titration with disodium dihydrogen ethylenediaminetetraacetate (tradename, Versenate) as outlined in U.S.D.A. Handbook No. 60 [4]. The calcium and magnesium in acetic-acid extracts were determined as before excepting that the interfering acetate ions and the soluble organic matter were first eliminated by treatment and evaporation with aqua regia as given in the Handbook. In the soils where soluble iron and aluminium were encountered, these were removed prior to calcium and magnesium estimation by complexing them with sodium diethyl dithiocarbamate and extracting off the complexes with iso-amyl alcohol. Insoluble carbonate was assessed by observing the degree of effervescence with dilute hydrochloric acid. Gypsum was qualitatively estimated by precipitation with acetone, but was found to be absent in all the soils examined.

EXPERIMENTAL RESULTS AND DISCUSSIONS

The results of the mechanical analysis of the surface soils together with one complete profile analysis are given in Table I. The silt plus clay percentage does not show a very wide difference and ranges from 70 to 90 per cent except at one place where it is 66.65 per cent. This is not very surprising since these soils were formed by the deposition of fine water-borne particles. The silt + fine particle load and the velocity of water carrying it must, however, have varied at different times and at different places. From the preponderance of fine sand over coarse sand, it can be inferred that deposition took place under conditions of very low water velocities, and that the water-borne material must have contained very little of the coarse fraction when these profiles were deposited.

TABLE I

Results of mechanical analysis of soils							
Sample No.	Depth in inches	Air-dry moisture %	Coarse sand %	Fine sand %	Silt %	Clay %	Textural designation
P234	0–12	2.74	Tr.	18.35	44.05	37.40	Silty clay loam
P238	0–12	2.68	Tr.	6.60	50.00	40.75	Silty clay
P239	12–24	2.65	Tr.	6.30	50.00	42.00	,,
P240	24–36	3.25	3.06	3.42	46.25	43.00	,,
P241	36–58	3.45	1.44	3.90	42.65	46.30	,,
P242	0–12	3.18	Tr.	3.84	52.50	41.00	,,
P246	0–12	3.52	Tr.	16.50	40.45	39.50	Clay loam
P295	0–12	2.67	Tr.	32.50	37.00	29.65	,,
P299	0–12	4.40	Tr.	22.24	40.70	33.55	,,

The results of analysis of the hydrochloric-acid extracts are given in Table II. From the data it can be seen that there is no striking variation in the figures obtained from the different samples. The R_2O_3 content does not increase with depth to any appreciable degree indicating that very little illuviation has taken place. This is to be expected considering the fact that these soils are of recent origin and consist primarily of unconsolidated alluvium with no distinct profile layers. The phosphate and iron contents of the soils are also quite high, giving rise to the belief that the phosphate is locked up in the strongly unavailable forms of iron phosphate.

TABLE II

Results of analysis of HCl extracts of soils							
Sample No.	Depth in inches	Fe_2O_3 %	Al_2O_3 %	R_2O_3 %	CaO %	K_2O %	P_2O_5 %
P234	0–12	5.04	5.57	10.61	0.66	0.55	0.160
P235	12–24	4.88	6.48	11.36	0.36	0.62	0.117
P236	24–36	5.88	4.27	10.15	0.36	0.75	0.067
P237	36–48	4.28	6.23	10.51	0.37	0.61	0.087
P238	0–12	6.40	8.46	14.86	0.77	0.54	0.120
P239	12–24	6.80	8.40	15.20	0.77	0.52	0.142
P240	24–36	8.00	7.84	15.84	0.91	0.45	0.113
P241	36–48	6.04	8.46	14.50	0.35	0.53	0.104
P242	0–12	5.52	6.40	11.92	0.42	0.61	0.123
P243	12–24	5.04	5.79	10.83	0.35	0.64	0.086
P244	24–36	4.20	6.89	11.09	0.32	0.58	0.083
P245	36–48	5.44	6.34	11.78	0.37	0.54	0.098
P246	0–12	8.08	7.06	15.14	0.42	0.47	0.153
P247	12–24	9.68	6.34	16.02	0.25	0.52	0.094
P248	24–36	9.72	12.28	22.00	0.31	0.41	0.094
P249	36–48	5.28	6.55	11.83	0.35	0.35	0.086
P295	0–12	4.16	6.86	11.02	0.78	1.40	0.173
P296	12–24	3.76	6.69	10.45	0.44	1.38	0.147
P297	24–36	3.84	6.84	10.68	0.69	1.41	0.162
P298	36–48	4.24	6.90	11.14	0.85	1.25	0.193
P299	0–12	5.76	6.76	12.52	1.44	1.27	0.143
P300	12–24	4.48	8.70	13.18	1.14	1.24	0.111
P301	24–36	5.68	8.88	14.56	1.31	1.14	0.119
P302	36–48	5.26	9.24	14.50	1.10	1.29	0.111

The pH, percent total soluble salts, carbon and nitrogen and C/N ratios of the soils are given in Table III. The pH of the soils varies from 4.9 to 8.6 and the percent total soluble salts from 0.081 to 1.580. A characteristic feature of some of these soils is the high percentage of organic carbon. Although conditions are not comparable, it is noteworthy that the carbon content is rather high compared with that of soils from other parts of Gangetic West Bengal. From the data given below, it can be seen that a low pH is associated with a high carbon content and vice versa. The nitrogen content is also high (compared with other areas in the state) and may be as great as 0.154 per cent. The C/N ratios indicate that humification has progressed considerably, and indeed the

values are somewhat lower than their counterparts in cool, temperate areas. Of course under tropical conditions, greater oxidation is apt to take place and thus the originally humus-rich layers become depleted of their organic matter content. Mohr [6] gives the impression that the presence of much iron furthers the direct or indirect oxidation of the organic matter in the soil, and since these soils contain appreciable amounts of iron as evident from Table II, it seems possible that humification has proceeded to an extent where the C/N ratio may not diminish any farther.

TABLE III

pH, percent total soluble salts, carbon and nitrogen and C/N ratios of soils

Sample No.	Depth in inches	pH	% T.S.S.	% C	% N	C/N
P234	0–12	5.95	0.081	0.930	0.122	7.7
P235	12–24	6.70	0.108	0.440	0.073	6.0
P236	24–36	5.30	0.042	0.714	0.084	8.5
P237	36–48	6.50	0.051	0.210	0.059	3.6
P238	0–12	8.00	0.460	0.330	0.050	6.6
P239	12–24	7.90	0.520	0.320	0.045	7.1
P240	24–36	5.00	1.200	0.550	0.058	9.5
P241	36–48	4.60	0.620	1.010	0.106	9.5
P242	0–12	7.50	0.144	0.710	0.099	7.2
P243	12–24	6.80	0.141	0.252	0.057	4.4
P244	24–36	6.70	0.158	0.258	0.050	5.2
P245	36–48	6.60	0.188	0.240	0.052	4.6
P246	0–12	4.90	0.512	1.050	0.154	6.8
P247	12–24	5.10	0.551	0.722	0.104	6.9
P248	24–36	5.30	0.704	0.585	0.089	6.6
P249	36–48	5.20	0.817	0.905	0.088	10.3
P295	0–12	6.40	0.603	0.593	0.074	8.0
P296	12–24	6.60	0.362	0.458	0.075	6.1
P297	24–36	6.70	0.141	0.186	0.034	5.5
P298	36–48	6.90	0.115	0.132	0.031	4.3
P299	0–12	8.60	0.904	0.424	0.099	4.3
P300	12–24	7.80	0.685	0.300	0.057	5.3
P301	24–36	7.50	1.126	0.360	0.050	7.2
P302	36–48	7.40	1.583	0.456	0.052	8.8

The data for cation-exchange capacity, acetic-acid-soluble and water-soluble cations are given in Table IV. The figures for exchangeable cations obtained by deducting the water-soluble cations from the acetic-acid-soluble cations are probably reliable in all

cases excepting P298, P299, P300, P301 and P302 where the presence of calcium and magnesium carbonates vitiates the results. The figures for exchangeable sodium and potassium closely approximate the actual amounts though in this case too, hydrolysis of some of the exchangeable sodium and potassium cannot entirely be ruled out. The exchangeable sodium percentage or the degree

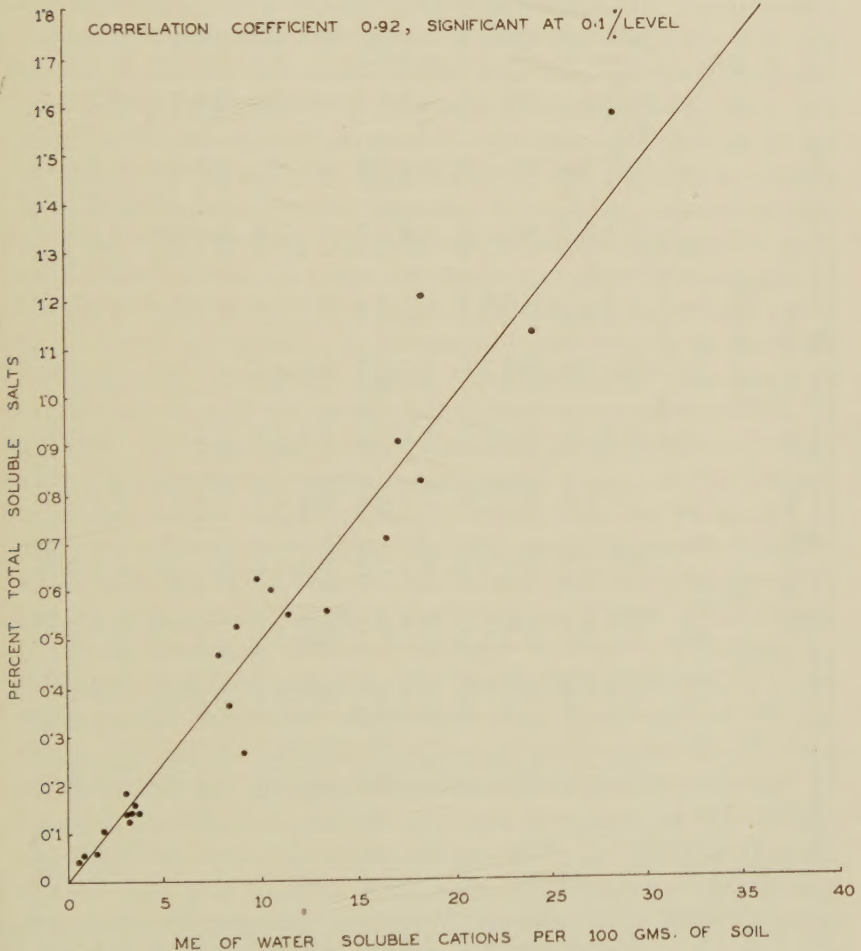

Fig. 1. Relationship between percentage total soluble salts and me of water-soluble cations per 100 g of soil.

of saturation of the exchange complex by sodium and the degree of calcareousness are also indicated in Table IV (column 17).

TABLE IV

Degree of calcareousness, cation exchange capacity, acetic-acid and water-soluble cations, exchangeable cations and exchangeable sodium percentage of soils.

Sample No.	Depth in inches	Degree of calcareousness	C.E.C.	HAc-soluble				Water-soluble				Exchangeable				Exchangeable Na percentage
				Ca	Mg	K	Na	Ca	Mg	K	Na	Ca	Mg	K	Na	
			me per 100 grams of soil													
P234	0–12	—	12.4	6.00	2.79	0.45	1.52	0.21	0.35	0.15	0.65	5.79	2.44	0.30	0.87	7.0
P235	12–24	—	16.2	5.46	3.99	0.72	2.91	0.35	0.35	0.24	0.76	5.11	3.64	0.48	2.15	13.3
P236	24–36	—	13.2	3.74	4.00	0.32	0.87	0.14	0.06	0.10	0.43	3.60	3.94	0.22	0.44	3.3
P237	36–48	—	14.4	5.68	4.83	0.51	1.09	0.21	0.08	0.12	0.48	5.47	4.75	0.39	0.61	4.2
P238	0–12	—	15.9	11.25	4.35	0.82	6.10	2.03	3.22	0.22	2.26	9.22	1.13	0.60	3.84	24.1
P239	12–24	—	14.4	11.55	6.40	0.81	6.33	2.52	3.50	0.22	2.37	9.03	2.90	0.59	3.96	27.5
P240	24–36	—	17.5	7.66	6.18	0.69	8.04	5.52	3.54	0.28	4.20	2.14	2.64	0.41	3.84	24.6
P241	36–48	—	16.7	2.55	6.61	0.64	9.52	1.19	3.99	0.26	4.00	1.36	2.62	0.38	5.52	33.1
P242	0–12	—	16.3	7.43	1.01	0.62	5.43	0.39	0.45	0.21	2.17	7.04	0.56	0.41	3.26	20.0
P243	12–24	—	15.5	5.88	1.86	1.13	7.17	0.37	0.41	0.46	1.96	5.51	1.45	0.67	5.21	33.6
P244	24–36	—	14.6	5.88	1.86	1.18	7.83	0.35	0.47	0.54	2.83	5.53	1.39	0.64	5.00	34.2
P245	36–48	—	13.7	6.10	2.34	1.05	7.61	0.42	0.45	0.41	3.04	5.68	1.89	0.64	4.57	34.1
P246	0–12	—	11.2	3.30	5.86	0.43	7.40	1.40	4.48	0.15	5.20	1.90	1.38	0.28	2.20	19.6
P247	12–24	—	11.8	3.00	7.62	0.62	8.80	1.26	5.81	0.21	6.10	1.74	1.81	0.41	2.70	22.3
P248	24–36	—	13.8	4.20	8.47	0.62	10.54	2.24	6.02	0.21	7.83	1.96	2.45	0.41	2.71	26.8
P249	36–48	—	12.6	5.80	9.23	0.61	9.00	3.78	7.42	0.10	7.00	2.02	1.81	0.51	2.00	15.9
P295	0–12	—	11.2	4.43	2.22	0.39	11.41	0.28	0.19	0.10	8.60	4.15	2.03	0.29	2.81	25.0
P296	12–24	—	13.6	4.60	2.44	0.87	10.76	0.42	0.35	0.26	6.30	4.18	2.09	0.61	4.46	32.8
P297	24–36	—	12.6	6.70	2.45	0.95	5.65	0.63	0.21	0.28	2.40	6.07	2.24	0.67	3.25	26.4
P298	36–48	+	11.7	14.50	0.99	1.08	5.22	0.42	0.14	0.36	2.20	14.08	0.85	0.72	3.02	25.8
P299	0–12	+	14.5	22.71	23.05	0.81	21.42	0.56	3.15	0.22	13.20	22.15	19.90	0.59	8.22	56.8
P300	12–24	+	13.1	27.30	7.90	1.23	20.02	0.56	0.77	0.31	12.00	26.74	7.13	0.92	8.02	61.2
P301	24–36	+	15.1	24.40	31.92	1.64	37.83	0.84	2.87	0.31	25.00	23.56	29.05	1.33	12.83	84.9
P302	36–48	+	15.5	23.80	7.06	1.85	39.61	2.24	1.75	0.56	27.90	21.56	5.31	1.29	11.71	75.5

+ = Slightly available

From the data given in Table IV, it can be seen that of the six profiles examined, one can be considered as normal, another as non-saline alkali and the remaining four as saline alkali. In so classifying the soils, the broad conditions of total soluble salts and exchangeable sodium percentage are used as the criteria. As will be shown later, these soils can thus be classified when the dominant soil-forming factors active here are further taken into consideration.

The two dominant agents in soil formation here are rain and sea water. As the high water-soluble and exchangeable sodium and magnesium contents clearly indicate, these soils have been subjected to the influence of sea water at sometime in the past and have so been rendered saline or saline alkali. Where the salts in the soil have been leached away by rainwater in conditions of high topography and good drainage, the resulting soil is either normal or non-saline alkali depending upon the presence or absence of calcium in the soil. Where the sea water has still some access to the land owing to the lower topography, to proximity to the sea, or to restricted drainage and consequent higher water-table, the resultant soil solution is not only rich in salt but sodium comprises more than 15 per cent of the exchange complex and thus gives rise to saline alkali soils. The presence of humus or raw organic matter further complicates the matter and gives rise to soils which can be classed as degraded saline alkali soils as different from de Sigmond's degraded alkali soils (Mohr [6]). Thus it can be seen that the dynamics of H and Na soils operate jointly in the formation of some of these soils.

The exchangeable sodium percentage in these soils varies considerably. It is approximately true that soil structure begins to be unfavourably influenced when more than 5 per cent of the exchangeable cations consists of sodium (Van Beekom *et al.*[9]). Due to their high salt content, the saline alkali soils do not show so harmful an effect of sodium on their structure as the others, although they swell when wet and can easily be puddled. Because of their high salt content, these soils are flocculated and readily permeable, whereas the downward percolation of water is comparatively slow in the case of non-saline alkali soils.

The impression gained from the low cation-exchange-capacity values of the soils is that the clay fraction is predominantly kaolinitic in character. This is of obvious advantage for although these soils tend to be heavy, leaching problems will nevertheless not arise

nor will they pose as serious a threat as in the case of those heavy soils where clay minerals of the montmorin series predominate. Thus we see that the adsorption complex is not entirely in accordance with the heaviness of the soil, and consequently, most of the salts present in the soil will occur dissolved in the soil water. This is an advantage when the salts have to be leached out. Also as clay minerals of the montmorin series do not seem to abound, the destructive influence of Na ions on soil structure may not be important.

Coming next to the organic-matter content of the soil, we encounter the presence of semi-decomposed organic matter in the soil. These deposits sometimes occur in the top or upper layers but they occur mostly at lower depths considerably beyond the plant-root zone. These soils are usually formed in areas where the land has subsided owing to the land masses sinking under their weight into the plastic semi-fluid mud below. This layer of fluid mud occurs at various depths (variously estimated at 10′–50′ below the surface) and has a tendency to get squeezed into the sea or nearby creek or any other outlet it can find when the soil mass above exerts downward pressure on it. The subsidence of the delta front has obviously handicapped the reclamation of the littoral tract. Such a subsidence is usual in the case of deltas, and even occurs in the Rhine delta of Holland. The high acidity of the soil resulting from the release and breakdown of organic acids from the decaying organic matter might have resulted in the breakdown of the sesquioxides into ferrates and aluminates, and at pH 4.9, we might expect Al and Fe ions to be present in amounts toxic to plants. The H ions thus liberated also play their role in gradually replacing the Na and other cations in the exchange complex. Thus side by side, the dynamics of Na and H soils operate jointly in the formation of these littoral soils.

From consideration of the per cent total soluble salts, pH, organic carbon, total water-soluble cations and exchangeable sodium percentage, it can be seen that a high degree of correlation exists between percent total soluble salts and the total water-soluble cations present in the soil (Fig. 1). Regarding pH and exchangeable sodium percentage, Fireman and Reeve [2] observed that the relationship between the pH value of a soil and its exchangeable sodium is not sufficiently close or consistent to be of

much diagnostic value in assessing the degree of sodium saturation from pH value alone. Similar views were expressed by Richards [8] who states that there does not appear to be any useful general relation between the exchangeable sodium percentage and the pH value of a soil. However, in a later study, Fireman and Wadleigh [3], while undertaking a statistical examination of the analytical records of a large number of soil samples, came to the conclusion that the exchangeable sodium percentage could be predicted from the pH values, although the exact exchangeable sodium content of the soils had no such relation. The Handbook [4] emphasizes the need for testing the reliability of this relationship fully before use on any given group of soil samples. Though Agarwal and Yadav [1] working with saline and alkali soils in the upper reaches of the Indian Gangetic alluvium in Uttar Pradesh found a good degree of correlation between pH and exchangeable sodium percentage, such correlation was lacking in the present soils; this is in accord with the work of Kelley [5] who has shown that the total absorbed sodium is not necessarily correlated with pH. On the other hand, the organic carbon content of the soil has a bearing upon the soil pH and this is specially true at the lower pH levels. This anomaly explains why the salt-affected soils pose additional problems in their behaviour from similar salt-affected soils occurring in the arid regions, thereby rendering the reclamation of these soils somewhat different from their counterparts in the drier regions of India and elsewhere.

Although this investigation deals primarily with soils of the estuarine area of Gangetic West Bengal, the results of the investigation apply to a far wider sphere. Not only will the results have some bearing upon other salt-affected, coastal, estuarine tracts of India and other tropical countries, but from them, different lines of approach to tackle the problem of soil salinity can also be worked out and put into operation. The harmful effects of excessive salt concentration in the soil upon plant growth is wellknown, and in tropical countries where the pressure of population on agricultural lands is high, the menace of salt must be overcome if agricultural expansion is to occur in the near future. The frequency with which lowlying coastal lands are subject to periodic inundation due to breaches in the existing embankments or for sundry other reasons has assumed alarming proportions, and apart from the fact that the existing levees, embankments and dykes need be strengthened, the attendant deterioration of soil conditions demands careful attention and should not be lightly brushed aside. Whereas it takes a rather short time to close a breach in the dyke, the harm done to the soil

by the ingress of saline water takes a considerably longer time to be overcome even if the adverse effects of excessive sodium in the soil are counteracted by the application of chemical amendments or green manuring crops and drainage facilities are available.

Coming next to the part of the reclamation work falling within the scope of this paper, the following things ought to be done.

Soil survey work has to be undertaken with the following objects in mind: to determine the nature and extent of the salinity problem in the area, to find out the occurrence, distribution and nature of organic matter in the soil and to obtain relevant idea about sub-soil conditions.

Fertilizer and manurial trials must be started at a few sites to obtain an idea of the magnitude and variation of the responses of crops to them. The possibility of improving the soil structure is a line of work which is important for salt-affected soils and must be explored by the application of compost, farmyard manure and chemical amendments and also by the growing of salt-resistant green manuring crops. The carbon/nitrogen ratio in the organic horizon of the soil has a satisfactory value and its suitability for use as a manure will also have to be examined by the addition of lime. This will help not only in oxidizing the raw humus thus eliminating its unfavourable effects but also in reducing the acidity of such soils. To ameliorate the deleterious effects of salts present in the soil and of the high content of sodium in the exchange complex, lime and gypsum and mixtures of them together with other chemical amendments will have to be tried. The use of these materials is also likely to improve the soil structure.

In the above experiments, crops now being grown by the farmers of the locality as well as alternative crops and crop rotations which might be more suitable for the areas will have to be tried. Other possibilities for enhancing the productivity of the soils will also have to be examined as information becomes available.

Irrigation and drainage are recognised to be very important aspects in projects of amelioration or reclamation, and it is imperative that work on these aspects be simultaneously undertaken if any headway is to be made.

SUMMARY

Six profiles from a representative area in the southern part of the deltaic region of Gangetic West Bengal, India, were examined. Of these, one can be termed as normal, another as non-saline alkali, and the remaining four as saline alkali. Of the saline alkali ones, there was one profile which had a rather low pH. The acidic condition of the soil is due to the presence of decaying organic matter in the soil giving rise to what has been termed as a degraded saline alkali soil. In designating the soils as such, the broad conditions of total soluble salts expressed as percentage of sodium chloride per hundred grams of soil and the exchangeable sodium percentage have been used as the criterion. The soils have thus been classified taking into consideration the dominant soil-forming factors active here. Remedial

measures to improve these soils include the incorporation of green-manuring crops and the application of soil ameliorants like lime, gypsum and sometimes other chemical amendments. The soils must be surveyed in order to delineate the types of salt-affected soils, and during reclamation, drainage and irrigation measures should not be lost sight of either.

ACKNOWLEDGEMENTS

My sincere thanks are due to Dr. J. N. Mukherjee, Scientific Advisor to the Govt. of West Bengal, Department of Agriculture, Animal Husbandry and Forests for his critical stimulus, to Dr. H. K. Nandi, Director of Agriculture, West Bengal for his kind permission to publish the results of this investigation, and to Dr. S. K. Mukherji, Agricultural Chemist, West Bengal for his help and co-operation. The assistance rendered by Messers P. Chakravarty, D. Roy, P. Gupta, P. Roy and A. Neogi of the All Bengal Reconnaissance Soil Survey Scheme in supplying some of the analytical data presented in this paper is also gratefully acknowledged.

First received February 17, 1958. Revised draft July 11, 1958

REFERENCES

1 Agarwal, R. R. and Yadav, J. S. P., Diagnostic techniques for the saline and alkali soils of the Indian Gangetic alluvium in Uttar Pradesh. J. Soil Sci. **7**, 109–21 (1956).
2 Fireman, M. and Reeve, R. C., Some characteristics of saline and alkali soils in Gem County, Idaho. Soil Sci. Soc. Am. Proc. **13**, 494–8 (1948).
3 Fireman, M. and Wadleigh, C. H., A statistical study of the relation between pH and the exchangeable sodium percentage of Western soils. Soil Sci. **71**, 272–85 (1951).
4 Handbook of Agriculture 1954. No. 60, U.S. Dept. Agr., U.S. Soil Salinity Lab. Staff. L. A. Richards, Editor (1954).
5 Kelley, W. P., Alkali Soils – Their Formation, Properties and Reclamation. Reinhold Publishing Corpn., New York, U.S.A. Pp. 56 (1951).
6 Mohr, E. C. J., The Soils of Equatorial Regions. Edwards Brothers, Inc., Ann Arbor, Michigan, U.S.A. (1944).
7 Piper, C. S., Soil and Plant Analysis. Interscience Publishers, Inc. New York (1950).
8 Richards, L. A., Chemical and physical characteristics of saline and alkali soils of Western United States. Trans. Intern. Cong. Soil Sci. 4th Congr. Amsterdam **1**, 378–83 (1950).
9 Van Beekom, C. W. C., Van den Berg, C., De Boer, Th. A., Westerhof, J. J and Zuur, A. J., Reclaiming land flooded with salt water. Neth. J. Agr. Sci. **1**, 1–31 (1953).
10 Van den Berg, C., The influence of adsorbed salts on growth and yield of agricultural crops on salty soils. The inundations of 1944–45 in the Netherlands and their effect on agriculture. Verslag. Landbouwk. Onderzoek. **58.5**. Pp. 118 (1952).
11 Zuur, A. J., Drainage and reclamation of lakes and of the Zuiderzee. Soil Sci. **74**, 75–89 (1952).

STUDY OF SOIL ALGAE

III. BIOASSAY OF SOIL FERTILITY BY ALGAE

by Y. T. TCHAN

Microbiology Laboratories, Faculty of Agriculture,
University of Sydney, N.S.W. Australia

GENERAL CONSIDERATIONS

The bioassay of soil fertility in its most general sense includes field trials, glasshouse experiments, and microbiological tests. The techniques used in such methods have been comprehensively summarised [4 5]. Lawrence [9], and Alvey [3] have indicated the difficulties and errors likely to be encountered in glasshouse experiments. The use of microbiological methods for assay of soil fertility has been reviewed by Wieringa [16] and Jensen [7]. Although it is not the intention of the writer to examine in detail any of the techniques published in recent years, some important points relevant to this paper will be discussed.

The microbiological methods which have been used so far in attempts to assess plant nutrients in soil are open to criticism on several grounds, each of which will be examined in some detail.

1. *Unsuitable reaction (pH) of the assay system*

Generally speaking, the media used for the assay have been adjusted to a favourable pH for the growth of the testing organism. This pH may not necessarily be similar to that of the soil being assayed. Where this was the case the reaction of the soil added to the culture fluid would tend to change toward that of the culture medium. Such changes in reaction may result in changes (increase or decrease) in the availability of nutrients compared with their availability at the reaction of the soil under field conditions. The problem is further complicated when the activities of the test micro-organisms bring about an appreciable modification of pH. Swaby *et al.*[6] gave a detailed account of the *Aspergillus* method and discussed the influence of the acidity produced by this mould. On the other hand when Na-benzoate is added as substrate in Wang's [15] *Azotobacter* method, metabolism

of the benzoate and consequent liberation of the Na-ion is accompanied by changes of pH in an alkaline direction (Tchan, unpublished data).

2. *The undesirable effects due to addition of organic matter other than that of the soil sample*

Most test organisms used have been heterotrophs. Therefore it was necessary in these cases to add extraneous organic matter (sugar or organic salt) to promote the growth. There are two major objections to such additions of organic matter:

a) They are likely to promote not only the growth of the test organism but also of many other zymogenous organisms which may compete with or even suppress the test organism (Acock [1], Nicholas and Fielding [10], Jensen [7].)

b) The growth of organisms in an organic medium is frequently accompanied by changes in redox potential which may be such that certain nutrients originally present in the oxidised form are changed to the reduced form. Therefore under such conditions the methods are not suitable for estimation of Fe and Mn (Swaby *et al.*[6]).

3. *The undesirable effects resulting from sterilization of the soil sample*

The objection under 2a noted above could be overcome by sterilizing the soil. There are serious objections to such treatment however:

a) Some of the nutrient elements contained in the tissues of the living soil organisms may become available when the cells are killed. The quantity of such nutrients is by no means negligible. Heat treatment may also modify the physico-chemical properties of the soil thereby resulting in the release or fixation of nutrients.

b) In the soil the amount of available nutrients represents the balance between those rendered unavailable due to absorption by organisms or fixation on the one hand and those released from the mineral and organic matter of the soil on the other hand. When a soil is sterilized, apart from any physico-chemical modifications which result, the available nutrients estimated after such treatment only represents those available at that particular time under rather unusual circumstances (Kauffman [8]). Estimations under such conditions give no information about the release or fixation during the experimentation, except in so far as this is brought about by the test organism. Such considerations may underly the discrepancies noted when sterilized soils have been used. (Nicholas and Fielding [10], Steenbjerg [11], Swaby *et al.*[6]).

4. *The unsuitability of the test organisms used*

Some test organisms do not have the same mechanisms as higher plants for uptake of the nutrients in soil. Swaby *et al.*[6] have already pointed out that *Aspergillus* obtains most of its trace elements from those in solution rather than by exchange processes. Kauffman [8] using a *Corynebacterium* for the estimations of soil P obtained lower values for P than when the

classical citric-acid extraction method was used. It seems that the *Corynebacterium* used only the soluble P but not the exchangeable P of the soil. Unfortunately the author did not mention any details on this point.

5. *Unsuitable growth rate of the test organisms in relation to the speed of soil processes*

Although rapid growth rate of micro-organisms represents an advantage of the technique this may in some cases become a disadvantage. The significance of this can be visualised in the following circumstances. When the nitrate of the soil has been recently washed out by rain, the soil will be regarded as being low in available N if a rapidly growing micro-organism is used for the estimation. The result would be different if a slow-growth test organism is used. In such a case, the organic matter would have time to be ammonified and nitrified thereby giving a higher value of available nitrogen which would be more in line with that likely to be obtained under field conditions; similar considerations may be equally applicable in the case of other elements. The use of a slowly growing test organism would therefore allow the biodynamic aspect of soil processes to be taken into consideration; this is liable to be overlooked when rapidly growing test organisms are used. A degree of inconvenience associated with the necessity of long incubation period must be therefore accepted.

From the considerations above, it is clear that no existing microbiological assay method can meet all criticisms. An entirely new approach has to be made, if a suitable technique is to be devised. To avoid these criticisms, outlined above, the technique to be adopted should fulfill the following conditions:

1. The pH of the soils being investigated must not be altered to any appreciable extent during the test.
2. Extraneous matter should not be added to the media.
3. The soil should not be sterilized.
4. The test organisms used should be physiologically as close to the higher plants as possible.
5. The test organisms should not have too rapid a growth rate.
6. The technique should be very easy to carry out and should not require any special apparatus. This particular point is important for the practical application of the technique, which should be usable not only by microbiologists but by all branches of workers concerned with soil fertility.

If one proceeds by a process of elimination; the first condition implies that the test organisms must be able to tolerate a wide range of pH conditions and bring about little change in the pH of

the culture medium during growth. This excludes organisms such as *Aspergillus, Beijerinckia, Azotobacter* and others.

The second condition would exclude all heterotrophic organisms including *Cunninghamella*.

Winogradsky [17] had pointed out that pure-culture studies in soil microbiology have less value compared with the studies involving the total flora. Since soils are biodynamically active, this also applies to microbiological tests for assessment of available soil nutrients. A mixed culture of algae would comply with these and the remaining four conditions and the floristic composition would not need to be defined. For example algae able to grow at the pH of a particular soil will, by natural selection form part of the microflora that grow during the test with that soil. The pH of the soil apparently remains unchanged during algal growth (1, above). Being autotrophs, addition of organic matter is not necessary (2, above). The use of a mixed algal inoculum avoids the difficulties associated with sterilization of the soil. Algae already present in the soil could be regarded as part of the inoculum (3, above). The use of a mixed algal inoculum also satisfies the 4th and 5th conditions and as is apparent from Table I, may form the basis of a suitable technique.

TABLE I

Assessment of the relative merits of the various methods used in the microbiological assay of available nutrients in soil.
A = accepted; R = rejected

Requirement	*Aspergillus*	*Beijerinckia, Azotobacter*	*Cunninghamella*	Soil respiration; Soil perfusion	Algae
No alteration of soil pH	R	R	R	A	A
No addition of organic matter	R	R	R	A	A
No sterilization of soil	R	R	A	A	A
Test organism should be as physiologically similar to higher plant as possible	R	R	R	R	A
Slow growth rate	R	R	R	A	A
No special apparatus required	A	A	A	R	A

MATERIALS AND METHODS

The method of Tchan [13] has been used: A complete mineral nutrient solution such as is used for growing higher plants can be used. The nutrient whose availability is being determined is omitted. A certain amount of the soil being tested is added to the solution in order that it may supply any of the omitted nutrient. The nutrient solution has a pH previously adjusted so that its reaction is similar to that of the soil under test. The response of a mixed algal inoculum introduced into the nutrient solution–soil mixture is ascertained by comparison with the growth in the control and in a series of cultures growing in the presence of a known amount of the nutrient whose level of availability in the soil is being determined.

Previously there have been difficulties in the way of assessing algal growth. Swaby *et al.*[6] have rejected the use of an alga as a test organism on the grounds of difficulty in measuring growth and the inability of the organism to demonstrate Mn and Mo deficiency. However, Swaby *et al.*[6] tried only one organism (*Chlorella vulgaris*) and did not investigate fully the means whereby algae might be used for this purpose. Several methods of assessing algal growth have been tried. Direct microscopy (Tchan [12]) is unsuitable because a fluorescence microscope is required and the counting is tiresome. The algal growth could be estimated indirectly from the amount of C assimilated. Preliminary work showed, however, that the increase in C resulting from the photosynthetic activity of the algae, although giving good results in the case of sandy mineral soils, was not large enough to give a reasonable accuracy in the case of soils rich in carbon. This technique was rejected because of these limitations. It is possible to use O_2 output as measured by means of a Warburg respirometer as a measure of algal population; together with the use of CO_2 containing isotopic C or the use of polarographic techniques, such measurements of algal growth are rejected on the ground of the delicate manipulations and expensive apparatus required. It would be possible to remove the O_2 by evacuating the system and then determine the amount of dissolved O_2 produced in the system during a short incubation period. This has no particular advantage in our case but it could be used for other purposes. It is possible, however, to assess the algal population from chlorophyll determinations.

The following technique proved to be satisfactory.

The method as used for the bioassay of available N has been chosen to illustrate the manner in which the technique is used. (More details of the method will be given in later papers).

The basic N-free medium is that described by Allen and Arnon [2].

(A) Major elements
- 0.001 *M* $MgSO_4$
- 0.005 *M* $CaCl_2$
- 0.004 *M* NaCl
- 0.002 *M* K_2HPO_4

(B) Trace elements	Fe-EDTA complex	4	ppm of Fe
	$MnSO_4.4H_2O$	0.5	ppm of Mn
	MoO_3	0.1	ppm of Mo
	$ZnSO_4.4H_2O$	0.5	ppm of Zn
	$CuSO_4.5H_2O$	0.02	ppm of Cu
	H_3BO_3	0.05	ppm of B
	NH_4VO_3	0.01	ppm of V
	$NiSO_4.6H_2O$	0.01	ppm of Ni

Some elements (Co, W, Ti, Cr,) included in the original medium have been omitted.

(C) NH_4NO_3 in various concentrations.

5–10 g of air-dried soil were added into 50 ml of the N-free medium. The pH of the medium was adjusted so that it was similar to the soil being tested. Replicate flasks containing different concentrations of N were prepared.

A mixed culture of soil algae was used as inoculum. It was cultivated in the basic medium to which 10 ppm of N had been added. The culture was filtered through loosely packed glass wool to obtain a homogeneous suspension. The algal cells were then separated and washed in N-free medium by centrifugation and finally resuspended in N-free medium. Two drops of this washed suspension were used for each flask.

The flasks were then incubated under a battery of fluorescent lamps (daylight warm-tint types) for 2 to 3 weeks.

Extraction of chlorophyll: The culture was filtered through No. 1 filter paper to separate the cells from the fluid. The filter paper was then returned to the flasks and immersed in 25 ml of commercial ethanol (also known as methylated spirit). A small amount of $CaCO_3$ was added to neutralise acidity derived from the cell extract and the acid soil, and also to flocculate the soil colloids. The flask was kept in a cool room overnight. The extraction of chlorophyll was completed by treatment with successive quantities of ethanol until the total volume reached 50 ml. The chlorophyll was estimated by its absorption at 6800 Å.

RESULTS

1. *Chlorophyll recovery test*

Two types of soil were used: (a) sandy soil; (b) clay soil with litter. The two soils were practically free of algae initially when checked by extraction and fluorescence microscopy examination. The results in Table II show the recovery rate as indicated by chlorophyll extractions, when known amounts of algal suspension are added to the two soils.

TABLE II

Chlorophyll recovery from added algae. (The optical absorption of the chlorophyll extracted from 1 ml of algal suspension is taken as 100)		
Medium	Optical absorption	Recovery rate as a % of chlorophyll added
Sandy soil	—	—
Clay soil	—	—
1 ml algal suspension	0.04	100
1 ml algal suspension + 5 g of sandy soil	0.04, 0.039	100, 97
1 ml of algal suspension + 5 g clay soil	0.0395, 0.0405	99, 101

2. *Exchangeable and soluble N and P in soil*

The type of the soil-N available to the algae was ascertained by the following simple experiments. 5 g of soil were placed in a dialysis bag and immersed in N-free basic medium. A control was provided by placing 5 g of soil in N-free medium to which a dialysis bag was also added. Two drops of washed algal suspension were added to each flask (outside the dialysis bag). After incubation for one week it became obvious that the algae could obtain exchangeable N from the soil since there was much better growth of algae in the control flask. After two weeks the flask having soil in a dialysis bag started to show some blue-green algae of the *Nostocaceae*: probably N-fixing types. This suggested that the soil used was poor in diffusable N since the control flask still contained a dominant flora of green algae.

The results were much more clear cut when similar experiments were carried out to assess the available soil-P. In an experiment in which P-free basic medium was used the flask with the soil contained in a dialysis bag gave a negligible growth of algae (Hawkes, unpublished data: based on colorimetric determination of extracted chlorophyll).

TABLE III

Utilisation of exchangeable phosphorus. The quantity of chlorophyll is expressed as optical absorption of light. (Data obtained by Hawkes in the author's laboratory)		
Experiment	Soil in dialysis bag	Soil outside the dialysis bag
1	0.02	0.049
2	0.013	0.046

3. *Growth of algae at different pH*

When the mixed algal culture was inoculated into media of different pH fairly satisfactory growth was obtained. When the pH is too acid to provide enough dissolved CO_2 it is advisable to use a small container with a carbonate-bicarbonate buffer.

4. *Determination of the quantity of soil which should be used in bioassay procedure*

The quantity of soil which should be used varies according to the nutrient element being investigated (*e.g.* 5 to 10 g per 50 ml of medium for N-assay purpose and 1 g per 100 ml of medium when P is being assayed). However the quantity of soil to be used for a particular element is determined by preliminary experiments, with several soils of known response to that element for higher plants. It is thus possible to ascertain the quantity of soil which would give the closest correspondence between the algal method and the higher plant response. This then becomes the quantity used for subsequent assays for the element concerned. The full details will be published in later papers.

5. *Duration of incubation*

Series of algal cultures were set up and the chlorophyll estimations carried out every 2 days. It was found that the amount of extractable chlorophyll increased rapidly during the first 8 to 10 days and then remained constant for about 3 to 4 days and thereafter diminished: *i.e.* 10 to 14 days could be regarded as a suitable incubation time. A similar conclusion was reached by Hawkes (unpublished data) with P.

6. *The influence of the N-content of the nutrient solution on algal growth*

Series of algal cultures were set up with different levels of N. The chlorophyll estimations were carried out after 11 days of incubation. Table IV summarises the results obtained.

TABLE IV

Influence of the concentration of N on the quantity of chlorophyll produced in culture (expressed as absorption)

Concentration of N in ppm	0	1	2	4	10	20	50
Chlorophyll produced	0.014	0.017	0.022	0.041	0.052	0.062	0.082
	0.015	0.018	0.019	0.042	0.047	0.065	0.080

7. Comparison between algal growth and plant responses

Oats were grown under pot-experiment conditions in a glass-house. Certain pots were watered with a complete nutrient solution and others were watered with a similar solution in which the N was omitted. The same soils were used for assays of available N by the algal method. The full details will be published in subsequent papers. Table V shows representative results obtained under the above-mentioned conditions.

TABLE V

Comparison of growth responses of algae and higher plants				
Soil No.	Test organism	Complete nutrient solution *	Nutrient solution —N. †	Difference between the growth response of higher plants and algae
3	Oats	100	15	9%
	Algae	100	24	
31	Oats	100	107	10%
	Algae	100	117	

* Growth reckoned as 100%.

† Growth expressed as a percentage of that obtained in complete nutrient solution.

DISCUSSION

The field trial seems to be the most acceptable test of soil fertility in the mind of agriculturists. All other fertility tests must be compared to or correlated with it. However the field trial is by no means constant or reproducible nor does it necessarily measure intrinsic nutrient status. The results obtained vary from season to season, from plant to plant and can be modified by other limiting factors than nutrient availability. On the other hand the bioassays carried out under constant conditions by micro-organisms are reproducible. Lack of correlation between the standardised laboratory test and field trials, subject to the hazards already noted, is not necessarily a reflection on the value of the laboratory test as a measure of intrinsic nutrient availability. The assessment of a soil's intrinsic fertility would for practical purposes remain an agronomic problem. For theses reasons if microbiological assays are to be used in agricultural practice they must be compared to some test other than the field trial. Pot experiments would appear

to provide the best means of comparison because they can be standardised more easily than field trials. The application of the results of bioassay tests to agricultural practice is dependent on their interpretation in relation to climatic and cultural conditions and the crop being grown.

Microbiological assays have some other advantages over pot experiments and field trials, *e.g.* less soil is needed (which can be important when the transport system is not adequate), and the results are obtained in a shorter time. However as indicated earlier the previous microbiological assay for soil-fertility tests are not satisfactory and open to serious criticisms. These criticisms are overcome by the technique in which a mixed algal culture is used.

The extraction of chlorophyll by ethanol is rather a crude method. However determinations of the absolute quantity of chlorophyll is not important. If the measurement did not give the theoretical accuracy in chlorophyll estimation, it gave a good agreement between duplicates and correlates well with higher-plant growth and these are more important considerations from the present point of view. Other ethanol-soluble substances were present in similar quantities in the control and N-added cultures. It is likely that the errors mentioned may cancel each other out and have no great effect on the final results. Other solvents have been tried (*e.g.* 80% acetone, fat solvents *etc.*) but they did not give any appreciable advantage, only additional complications. More accurate measurement could theoretically be obtained by measuring the fluorescence of the chlorophyll extracts. This procedure may be theoretically attractive but certainly has to be rejected because of the 6th condition mentioned above.

Algae and higher plants show similar growth responses in the presence of different quantities of N. Theoretically, it would be possible to apply the principal of the Mitscherlich method and its application should be kept in mind when the present technique has been sufficiently tested in the field. A large increase in algal growth on addition of a particular nutrient to the test system indicates a deficiency of that nutrient in the soil. One can easily classify the soil samples as very deficient, deficient, moderately deficient, and not deficient. Such a classification would be adequate for agricultural practice. For more precise work it is only necessary to express the results as a percentage of the growth obtained in the

presence of the complete nutrient solution (reckoned as 100%). The method has no pretensions of giving quantitative estimations of nutrient elements. It aims rather at predicting the response by plants when fertilizer is supplied to a particular soil. This is one of the major aims of agriculture.

The technique itself is simple enough to be carried out by anyone with a minimum training. Since pure cultures are not used the normal bacteriological precautions are not needed. The tests can be carried out in any laboratory, experimental station or farm, with a minimum of chemicals and glassware. Visual comparison of the green colour of ethanol extract gives a good indication of the amount of algal growth, when only large responses are being looked for. The use of a colorimeter is not necessary. One can easily use an arbitrary scale of green solution and obtain a semi-quantitative result.

A constant-temperature room or incubator is desirable but not essential. Since all the cultures of a particular test are submitted to the same variations in temperature, the results are comparable among themselves. It is more important that all the test cultures should receive the same amount of light. However, any place, except the direct sunlight can be used if one takes the precautions of providing a uniform light intensity over the whole area. (A photographic exposure meter checking the evenness of the illumination is adequate for general purposes).

Compared with the chemical analysis of soil, the algal culture method does not take more time or labour. All the reagents (nutrient solutions) can be stored as concentrated solutions. They then only require dilution before use. The incubation period seems long but the tests require practically no attention during this time.

The algal assay technique may be criticised on the ground that the soil is immersed in nutrient solution, but this is partly offset by production of O_2 by algae. No appreciable local reduction takes place. In fact the soil under these conditions is much less subject to reducing conditions than a soil *in situ* saturated with water. One can overcome such difficulties by replacing the liquid medium by silica gel or pure sand but since the response obtained by the present technique seems satisfactory this complication is not necessary.

To conclude the discussion, the statement by Waksman [14] will

be considered. Waksman stated: "The major difficulty involved in the use of such methods was the reactions brought about by micro-organisms in soil under natural conditions are subject to too many variables. These comprise not only inherent differences in soil conditions, but also the effects of climate and soil management... As long as knowledge of the effects of these variables was lacking, the information obtained by microbiological procedures was limited in scope and had little application to practical agriculture". Such a statement could be taken to mean that all microbiological methods of assessing available soil nutrients are unsatisfactory if it is not appreciated that the same statement is equally applicable to other methods of determining availability of soil nutrients such as chemical analyses, pot experiments including those of the Mitscherlich type, field trials and seedling method of Neubauer. If it were accepted, such an outlook would be misleading for the whole field of nutrient-availability determination in soil and prevent progress towards the development of any method whose results could be satisfactorily applied in agriculture practice.

SUMMARY

1. A number of criticisms can be levelled at the existing methods of microbiological assay of available soil nutrients. These criticisms are examined in some detail.

2. A new method for the microbiological assay of available soil nutrient is described using algae as the test organisms.

3. The new technique overcomes the criticisms levelled at previous methods.

4. The simplicity of the method makes it suitable for general use.

ACKNOWLEDGEMENTS

The author would like to thank Professor J. M. Vincent and Dr. J. Bunt (of Sydney University) for criticisms of the manuscript. He also wishes to thank Mr. R. Hawkes and Miss E. Jackson for help with the experimental work and the use of data.

Received June 11, 1958

REFERENCES

1 Acock, A. M., An examination of Mulder's rapid biological method for estimating the amount of available copper in soils. J. Council Sci. Ind. Research **14**, 288–300 (1941).

2 Allen, M. B. and Arnon, D. I., Studies on nitrogen-fixing blue-green algae. 1. Growth and N-fixation by *Anabaena cylindrica* (Lemm.). Plant Physiol. **30**, 366–372 (1955).

3 Alvey, N. G., Error in glasshouse experiments. Plant and Soil **6**, 347–559 (1955).

4 Demolon, A., La dynamique du sol. Dunod, Paris (1944).

5 Diagnostic techniques for soils and crops. American Potash Institute. Washington 6 D.C. (1948).

6 Donald, C., Passey, B. I., and Swaby, R. T., Bioassay of available trace metals from Australian soils. Australian J. Agr. Research **3**, 305–325 (1952).

7 Jensen, H. L., Microbiological determination of micro-nutrients in soil. Suomen Kemistilehti *A* **24**, 197–203 (1951).

8 Kauffman, J., Méthode pratique de dosage microbiologique du phosphore. Application au dosage du phosphore dans les terres. Ann. Inst. Pasteur **85**, 247–253 (1953).

9 Lawrence, W. J. C., Techniques for experiments with pot plants. Plant and Soil **6**, 332–346 (1955).

10 Nicholas, D. J. and Fielding, A. H., The use of *Asp. niger* (M) for the determinations of magnesium, zinc, copper and molybdenum available in soils to crop plants. J. Hort. Sci. **26**, 125–57 (1951).

11 Steenbjerg (see Jensen [7]) (1940).

12 Tchan, Y. T., Study of soil algae 1. Proc. Soc. Linn. N.S.W. **77**, 265–269 (1953).

13 Tchan, Y. T., Estimation of nutrient elements in soil by algae. Principle of the method. Congr. Intern. Sci. Sol Paris **C**, 249–251 (1956).

14 Waksman, S. A., Soil Microbiology, p. 326–327. John Wiley and Sons Inc. (1952).

15 Wang, T. L., A new method for determining the fertilising mineral elements of soil by Azotobacter. Plant and Soil **5**, 41–46 (1951).

16 Wieringa, K. T., Determination of the fertility of the soil by microbiological methods. Antonie van Leeuwenhoek **61**, 57–70 (1939).

17 Winogradsky, S., Microbiologie du sol. Masson et Co. Edit. (1949).

Plant and Soil X, no. 3 March 1959

EFFECTS OF BICARBONATE AND SOME OTHER ANIONS ON THE SHOOT CONTENT OF P^{32}, Ca^{45} Fe^{59}, Rb^{86}, Sr^{90}, Ru^{106}, Cs^{137}, AND Ce^{144} IN BEAN AND BARLEY PLANTS *

by JAMES A. GOSS ** and E. M. ROMNEY

Department of Botany and Atomic Energy Project,
University of California, Los Angeles

INTRODUCTION

The presence of high levels of Ca in certain soils is known to inhibit the absorption of Sr by plants [22]. This effect is attributable mainly to the complementary relationships between the Sr and Ca cations, although other factors might be involved. For example, the high contents of bicarbonate anion that are found in calcareous soils might inhibit the absorption of Sr (and other important long-lived fission products) by plants, since bicarbonate has been found to affect the mineral status of plants [3 6 9 10 12 14 18 24 26 27].

The purpose of this paper is to report on investigations which have been carried out to elucidate the role of the bicarbonate ion as it may affect the movement of mineral elements from the substrate into the plant shoot. The radioactive tracers P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, Sr^{90}, Ru^{106}, Cs^{137}, and Ce^{144} were used in these studies.

EXPERIMENTAL METHODS

Bean (*Phaseolus vulgaris*), var. Six-weeks Bountiful, was selected as the primary indicator plant. Selected treatments were extended to barley plants

* This work was conducted under contract AT-04-1-GEN-12 between the University of California and the Atomic Energy Commission, and the data in this paper were partially taken from a dissertation presented by J. A. Goss to the Graduate School of the University of California at Los Angeles in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

** Present Address: Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

(*Hordeum vulgare*), var. Atlas, to observe the treatment effects between different plant genera. The experiments were carried out in a semi-controlled environment. The light source consisted of fluorescent tubes and Mazda bulbs which produced a light intensity of about 800 foot candles at the level of the plant shoots. The air was filtered to remove toxic gases. The air temperature was not controlled, but it remained at 30° plus or minus 5° C.

Ten-day old bean seedlings were transferred to 3 liters of aerated nutrient solution containing 5×10^{-3} *M* $Ca(NO_3)_2$, 3×10^{-3} *M* $MgSO_4$, 1×10^{-3} *M* KH_2PO_4, 2×10^{-3} *M* KNO_3, and 2×10^{-3} *M* K_2SO_4. Micronutrients were added at levels of 0.5 ppm B, 0.4 ppm Mn, 0.5 ppm Zn, 0.02 ppm Cu, and 0.01 ppm Mo. Iron was added as the chelated Fe-EDTA at 2.5 ppm Fe. Commercial distilled water was used in the preparation of all solutions. The initial pH was adjusted to pH 5.7–6.0 with dilute KOH solution. This standard nutrient solution was used as the control, against which all data were compared. The root temperature was maintained at 25° plus or minus 1° C by a controlled-temperature water bath into which the jars containing the nutrient solutions were placed. The standard nutient solution was renewed on the sixth day, and again on the eighth day at which time the treatments were applied. These consisted of the standard nutrient solution and ones to which NaCl, $NaNO_3$, Na_2SO_4, or $NaHCO_3$ was additionally applied at 10 me per liter of standard nutrient solution. This level was selected as the result of preliminary studies with the bicarbonate anion. All solutions were adjusted to a pH value of 8.1 with NaOH. The $NaHCO_3$ was added only after the pH value had been raised above 7.9. Immediately thereafter, 5μc of P^{32}, Ca^{45}, Sr^{90}, Ru^{106}, Cs^{137}, or Ce^{144}, 2.84 μc of Fe^{59}, or 10 μc of Rb^{86} was added per culture. Stable Sr was added to all Sr^{90}-treated cultures at 0.5 me $Sr(NO_3)_2$ per liter of solution; RbCl was added to all Rb^{86}-treated cultures at 0.14 me per liter of nutrient solution. No stable salts of Ru, Cs, or Ce were added other than the minute amounts present in the processed isotope solutions received from Oak Ridge, Tennessee. Combinations of P^{32} and Ca^{45}, as well as Ca^{45} and Sr^{90} were applied as double tracers. A close correlation of the Ca^{45} data obtained from these double-tracer experiments showed good agreement among replicates and favorable reproducibility between experiments. Each treatment was replicated six times and each replicate contained two plants per culture. The plants were harvested 8 hours after first exposure to the treatments. The shoots were removed above the cotyledonary node, in the case of the bean plants, weighed, oven-dried at 70° C, and ground by Wiley mill for radio-assay.

Barley seeds were germinated on cheese-cloth disks which were stretched over 7-inch wooden rings. These germination rings were floated in tap water under constant light. After germination, the seedlings were thinned to 50 per cheese-cloth disk. Five-day old seedlings were transferred to the standard nutrient solution and then treated as described for beans. Only P^{32}, Ca^{45} and Rb^{86} indicator isotopes were used for the barley experiments. Each replicate contained 50 barley plants per culture.

Duplicate, 500-milligram samples of oven-dried tissue were counted with an end-window G–M tube (1.9 mg/cm^2). Appropriate corrections were made

for backscatter, self-absorption, decay, and filter absorption. For double tracer counting, the Ca^{45} emissions were absorbed with a No. 11 aluminum filter (217 mg/cm). No assurance could be made that the specific activity relationships remained constant under the conditions of these experiments inasmuch as a precipitate was formed in the solutions at high pH. Therefore, no attempt was made to assess total elemental uptake by tracer measurements. Data represent the content of these radioactive tracers in the plant shoots in terms of disintegrations per second, as the percentage of the amount of radioisotope in the shoots of plants in the standard nutrient cultures.

EXPERIMENTAL RESULTS

Nutrient-solution considerations

The bicarbonate anion is an ionic species of the carbonic-acid system. At pH levels above pH 8.0, more than 95 per cent of this system exists as the bicarbonate anion [25]. For this reason the pH levels of the nutrient solutions were adjusted to pH 8.1. It was observed that a white precipitate formed in all nutrient solutions at this pH level. Specific-conductivity measurements, which were started within 3 minutes after the pH was adjusted and continued at various intervals up to 8 hours, indicated that the precipitate formed rapidly at this pH level in both the $NaHCO_3$ and the standard-nutrient treatments. Infra-red spectra and x-ray diffraction analyses on samples of the precipitates isolated after 8 hours following pH adjustment showed them to consist largely of amorphous tricalcium phosphate with an additional small amount of magnesium carbonate formed in the presence of added bicarbonate. Only small amounts of Fe were found associated with the precipitates.

Effect of treatment on P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, Sr^{90}, Ru^{106}, Cs^{137}, and Ce^{144} content of the shoots

Figs. 1 and 2 illustrate the effects of equivalent concentrations of NaCl, $NaNO_3$, Na_2SO_4 or $NaHCO_3$ on the shoot content of these radioisotopes in bean and barley plants. Data are expressed as per cent of the shoot content compared to the standard-nutrient treatment. The results show a marked inhibition of the bicarbonate anion on the shoot content of P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, and Sr^{90}, and this inhibition was significant at the 1 or 5 per cent probability level. The content of Cs^{137} was not reduced, and the reduction in the

uptake of Ru^{106} and Ce^{144} may or may not have been attributed specifically to the bicarbonate anion. Since these were supplied only in minute amounts, these results may be greatly influenced by reduced mass-action effects.

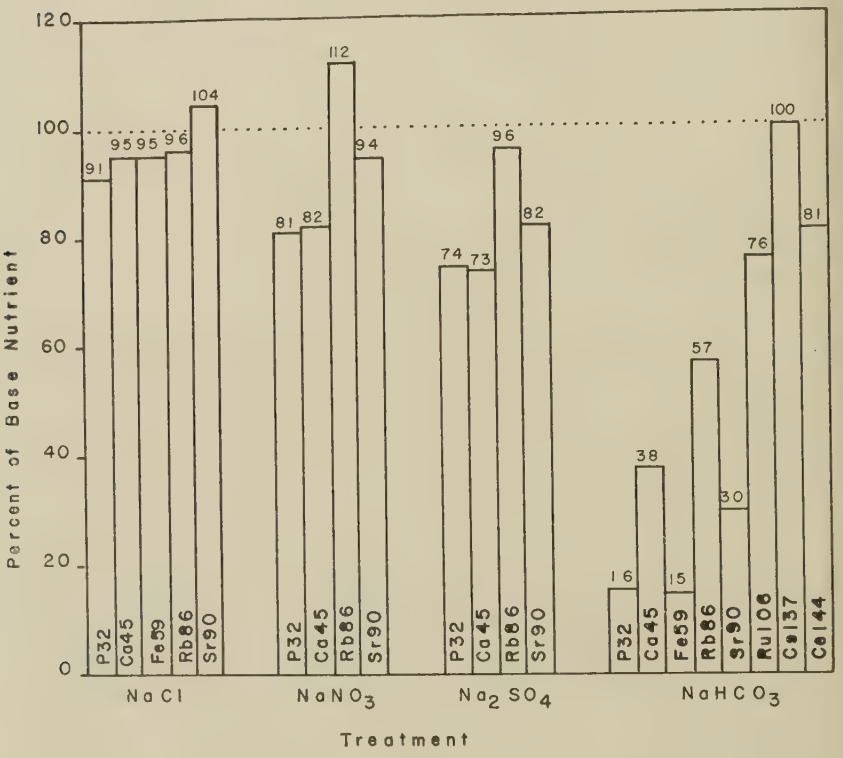

Fig. 1. Effect of treatment on the uptake of isotopes to bean shoots. The uptake is expressed as per cent of that from in the control base-nutrient treatment, with the percentage given at the top of each bar.

The inhibition was more severe in bean plants, particularly for Fe^{59} and P^{32} content. Significant inhibition at the 5 per cent probability level was observed for P^{32} and Ca^{45} content of bean shoots from Na_2SO_4 treatments. This was also the case for Ca^{45} content of barley from NaCl, $NaNO_3$, and Na_2SO_4 treatments. The Na_2SO_4 treatment significantly reduced the Rb^{86} content of barley shoots. The reduced content associated with the NaCl,

$NaNO_3$, and Na_2SO_4 treatments probably reflects the combined influence of the Na-ion activity as well as the anion activity. The bicarbonate treatment produced nearly the same inhibiting effects on both Ca^{45} and Sr^{90}, which are representative of the divalent cations, but it was less effective in reducing the shoot content of

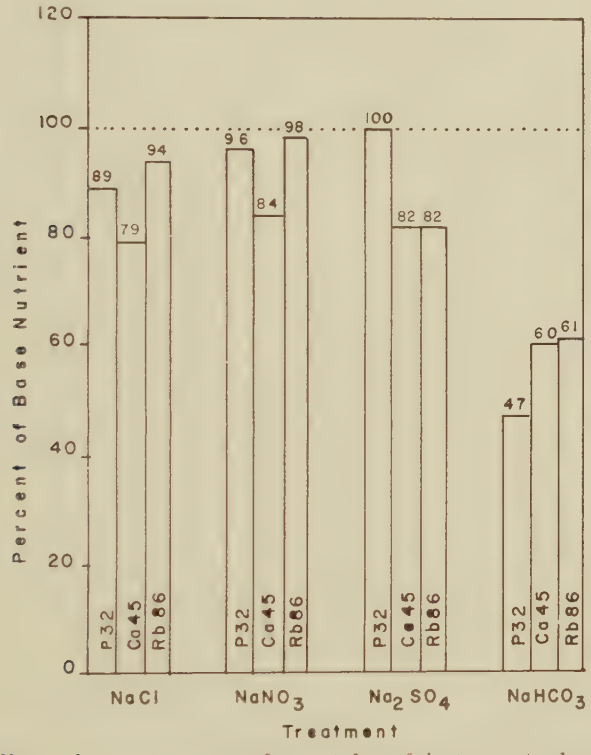

Fig. 2. Effect of treatment on the uptake of isotopes to barley shoots. The uptake is expressed as per cent of that from in the control base-nutrient treatment, with the percentage given at the top of each bar.

the monovalent Rb^{86} ion by bean leaves. Barley appeared to be more tolerant of the bicarbonate anion with reference to the amount of tracer in the shoots.

DISCUSSION

Differences in tracer content between bean and barley shoots generally were greater than differences attributable to any of the

treatments other than the bicarbonate anion. Barley was more resistant to the inhibiting effects of bicarbonate which gives a suggestion as to why barley can be grown more successfully than beans on alkaline-calcareous soils.

In addition to the inhibiting influence of calcium on strontium uptake by plants that is known to occur in calcareous soil or in acidic soil to which liming materials have been incorporated [22], the inhibiting effect of bicarbonate on Sr^{90} content observed in this study indicates that the high concentration of bicarbonate anion generally found in these types of soil may be partly responsible for the reduced uptake of strontium by plants.

A number of reports on the effects of bicarbonate on plants have emphasized the increased K/Ca ratio in the leaf tissue of some plants exposed to bicarbonate treatments. Our results with bean plants showed that the Rb^{86} content was reduced less than Ca^{45} content in the presence of added bicarbonate. Since Rb and K uptake by plants has been reported to occur at a similar rate, an increased K/Ca ratio may be assumed for the bicarbonate-treated bean plants. This would not appear to be the case for barley inasmuch as both Rb^{86} and Ca^{45} were reduced the same amount by the bicarbonate treatment [16].

The reduction in the Fe content of bean shoots is in agreement with the work of others [13 20]. The accumulation of ions other than Fe also was decreased by the bicarbonate anion; therefore, the direct effect of the bicarbonate may not be related specifically to Fe, as has been suggested by some investigators, but the reduced accumulation of other mineral nutrients must also be considered.

Phosphorus presents a special problem not found with certain of the other ions. The ionic species of orthophosphate are present in concentrations which vary with the pH; the HPO_4^{--} ion being prevalent at the higher pH values. A number of investigators have shown that the uptake of phosphorus by plants decreased at pH levels above 7 [8 18]. If it is assumed that both HPO_4^{--} and $H_2PO_4^-$ ions are taken up by plants, the reduced uptake of phosphorus may be explained by 1) the difference in the rate of uptake of each of the two species, or 2) that both are taken up independently and inhibited competitively by the OH^- ion. The first view is upheld by McGeorge [15], Arnon *et al.*[2], Olsen [19], and Arnon [1]; however, Hagen and Hopkins [8] presented evidence that both are absorbed

although their absorption is inhibited competitively by hydroxyl ions. Irrespective of the point of view taken, phosphorus uptake decreased with increasing pH, and since all of the treatments were at the same pH value, the ionic species present would be expected to be similar in all cultures. Buehrer [4] pointed out certain effects of the bicarbonate ion on the ionic species of P in solution that might affect phosphorus uptake by plants; however, the marked inhibition observed in our investigation is believed to be correlated largely with bicarbonate—plant interactions.

When looking for the cause of the bicarbonate effects on plants, we might consider the action of bicarbonate-produced CO_2. Either CO_2 or H_2CO_3 or both are known to enter plant cells quite readily [11 23]. However, there appear to be two separate mechanisms involved with the bicarbonate and CO_2 molecules [11 24 20]. How the bicarbonate functions within the cytoplasm – which has a pH of about 6.8 7.0 [5 23 28] – to bring about the inhibiting effect on the uptake of mineral nutrients to plant shoots is unknown. Some investigators have concluded that the carbon derived from bicarbonates can be assimilated into the organic-acid fraction of plant root cells [7 21 12]. Miller and Thorne [17] present evidence that the bicarbonate ion is associated with a decrease in the rate of respiration by excised root tips. Since respiration is associated with the uptake of mineral nutrients by plants, it might be expected that the bicarbonate decreases the rate of respiration, and thereby the rate of uptake. However, an apparent anomaly to this lies in the observation that the concentration of Ca, P and Fe which are associated with the roots, increases in the presence of added bicarbonate [19 18 20]. It therefore would appear that the bicarbonate ion is more closely involved in the translocation of minerals to the shoots than in mineral absorption.

SUMMARY

Bean and barley plants were cultured in nutrient solutions and subsequently treated to include a control and 10 me of NaCl, $NaNO_3$, Na_2SO_4, or $NaHCO_3$ added per liter. The content of P^{32}, Ca^{45}, Fe^{59}, Rb^{86}, Sr^{90}, Ru^{106}, Cs^{137}, or Ce^{144} in the shoots was determined after an 8-hour period on treatment.

The presence of added bicarbonate very significantly reduced the content of all radioisotopes studied except Cs^{137}. This reduction was more severe in

bean plants, particularly for P^{32} and Fe^{59}. A slight reduction was observed in the content of P^{32} and Ca^{45} in beans cultured on $NaNO_3$-treated solutions and of P^{32}, Ca^{45}, and Sr^{90} by beans cultured on Na_2SO_4 treatments. The shoot content of Ca^{45} was reduced in barley cultured on NaCl, $NaNO_3$, and Na_2SO_4 treatments. Barley appeared to be more tolerant of the bicarbonate anion than was the bean plant.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. Karl C. Hamner for his encouragement which made the completion of this study possible, and also for his review of the paper, as well as to Mrs. Lucille Goss for typing the manuscript.

Received July 8, 1958

REFERENCES

1 Arnon, D. I., The physiology and biochemistry of phosphorus in green plants. *In*: Soil and Fertilizer Phosphorus in Crop Nutrition, W. H. Pierre and A. G. Norman, Ed. Pp. 1–42. Academic Press, Inc. N.Y. (1953).

2 Arnon, D. I., Fratzke, W. E., and Johnson, C. M., Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiol. **17**, 515–524 (1942).

3 Brown, J. W. and Wadleigh, C. H., Influence of sodium bicarbonate on the growth and chlorosis of garden beets. Botan. Gaz. **116**, 201–209 (1955).

4 Buehrer, T. F., The physico-chemical relationships of soilphosphates. Ariz. Univ. Agr. Expt. Sta. Tech. Bull. **42**, 153–212 (1932).

5 Chambers, R., Micrurgical studies on propoplasm. Biol. Rev. Cambridge Phil. Soc. **24**, 246–265 (1949).

6 Gauch, H. G. and Wadleigh, C. H., Salt tolerance and chemical composition of Rhodes and Dallis grasses grown in sand culture. Botan. Gaz. **112**, 259–271 (1951).

7 Graf, G. E. and Aronoff, S., Carbon dioxide fixation by roots. Science **121**, 211–212 (1955).

8 Hagen, C. E. and Hopkins, H.T., Ionic species in orthophosphate absorption by barley roots. Plant Physiol. **30**, 193–199 (1955).

9 Heller, V. G., Hageman, R. H., and Hartman, E. L., Sand culture studies of the use of saline and alkaline waters in greenhouses. Plant Physiol. **15**, 727–733 (1940).

10 Hoagland, D. R., The absorption of ions by plants. Soil Sci. **16**, 225–246 (1923).

11 Jacobs, M. H., The production of intracellular acidity by neutral and alkaline solutions containing carbon dioxide. Am. J. Physiol. **53**, 457–463 (1920).

12 Kadry, L. T., Soil-plant relations of the bicarbonate anion to lime-induced chlorosis. Ph. D. thesis, Dept. of Soil Chemistry, Utah State Agr. College (1955).

13 Lindsay, W. L. and Thorne, D. W., Bicarbonate ion and oxygen level as related to chlorosis. Soil Sci. **77**, 271–279 (1954).

14 Lunt, O. R., Kohl, H. C., and Kofranek, A. M., Effects of bicarbonate and other constituents of irrigation water on the growth of azaleas. Proc. Am. Soc. Hort. Sci. **68**, 537–544 (1956).

15 McGeorge, W. T., Electrodialysis as a measure of phosphate availability in soils

and the relation of soil reaction and ionization of phosphate to phosphate assimilation. Ariz. Univ. Agr. Expt. Sta. Tech. Bull. **38**, 592–630 (1932).

16 McGeorge, W. T., Nutrient interrelations in lime-induced chlorosis as revealed by seedling tests and field experiments. Ariz. Univ. Agr. Expt. Sta. Bull. **116**, 295–338 (1948).

17 Miller, G. W. and Thorne, D. W., Effect of bicarbonate ion on the respiration of excised roots. Plant Physiol. **31**, 151–155 (1956).

18 Olsen, C., The significance of concentration for the rate of ion absorption by higher plants in water culture. IV. The influence of hydrogen ion concentration. Physiol. Plantarum **6**, 848–858 (1953).

19 Olsen, S. R., Inorganic phosphorus in alkaline and calcareous soils. *In*: Soil and Fertilizer Phosphorus in Crop Nutrition, W. H. Pierre and A. G. Norman, Ed. Pp. 89–122. Academic Press. Inc. N.Y. (1953).

20 Porter, L. K. and Thorne, D. W., Interrelation of carbon dioxide and bicarbonate ion in causing plant chlorosis. Soil Sci. **79**, 373–382 (1955).

21 Rabinowitch, E. L., Photosynthesis and Related Processes. II. Interscience Publishers, Inc. N.Y. (1956).

22 Romney, E. M., Effects of calcium and strontium on plant uptake of Sr90 and stable strontium from nutrient solutions and soils. Univ. Calif. Los Angeles. Report 374. (1956).

23 Small, J., Modern Aspects of pH. Bailliere, Tindall and Cox, London. (1954).

24 Steward, F. C. and Preston, C., Effects of pH and the components of bicarbonate and phosphate buffered solutions on the metabolism of potato discs and their ability to absorb ions. Plant Physiol. **16**, 481–519 (1941).

25 Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism. Burgess Publ. Co., Minneapolis. (1949).

26 Wadleigh, C. H. and Brown, J. W., The chemical status of bean plants afflicted with bicarbonate-induced chlorosis. Botan. Gaz. **113**, 373–392 (1952).

27 Wadleigh, C. H. and Brown, J. W., Influence of sodium bicarbonate on mineral composition of red garden beets. Proc. Am. Soc. Sugar Beet Technologists **54-57** (1952).

28 Wildman, S. G. and Cohen, M., The chemistry of plant cytoplasm. *In*: Handbuch der Pflanzen Physiologie, Springer Verlag, Berlin, 243–300 (1955).

Plant and Soil X, no. 3 March 1959

SOME FACTORS INFLUENCING CATION UPTAKE BY EXCISED ROOTS OF PERENNIAL RYE GRASS (*LOLIUM PERENNE*)

by RODERICK P. MURPHY *

Soil Laboratory, Johnstown Castle, Wexford, Ireland

INTRODUCTION

A number of mechanisms have been postulated to explain the uptake by plant roots of cations against apparent concentration gradients [1 3]. One of the most widely accepted of these involves the following reaction: –

$$HR + K^+ \leftrightarrows KR + H^+$$

where HR is a cation carrier produced by a metabolic reaction in some region of the plant cell. In connection with some work being done in this laboratory on the ability of certain types of plant roots to absorb nutrients from both culture media and soil, it was decided to examine this reaction. It was assumed that being a reversible reaction, it obeys the law of Mass Action *i.e.*, it can be driven either to the right or to the left by varying the concentrations of the hydrogen ion in the culture medium. As this work was ultimately to be related to the relatively complex solutions found in soil, the uptake experiments were conducted in a solution similar in nature to that in which the plant roots were grown.

METHODS

The material used was excised roots of perennial ryegrass (Westernwolths var.). The plants were cultured on Pyrex glass dishes (26 cm × 18 cm × 5 cm) across the top of which a grid made of Pyrex rods was laid. Over this and dipping into the trays at each end, was a sheet of butter muslin on which about 50 g of seeds were placed. The trays were filled with culture medium to within 0.5 cm of the top and the whole placed in the incubator at 26° C.

* Present address: Dept. of Pathology, St. Finbarr's Hospitals, Cork, Ireland.

The plants were allowed to grow for 5 days. At the end of this period the roots had grown to about 6 cm in length. On the day of the experiment these were excised as close to the seeds as possible, washed in distilled water, blotted between sheets of filter paper to remove excess moisture and weighed in 2-g lots. The proportion of 2 g excised roots to 40 ml absorption solution was maintained in all experiments done. The absorption solution was prepared so that it contained the following molar concentrations: —

Ions	Millimoles per litre
Ca^{++}	2
Mg^{++}	1
K^{+}	2.5 (approx.)
NH_4^{+}	2
NO_3^{-}	8
PO_4^{---}	2

The calcium, magnesium and ammonium ions are added as their nitrates and the phosphate is added as orthophosphoric acid. This gives a solution of approx. pH 3.2. The pH is raised to the required level with KOH and sufficient KCl added to bring the potassium concentration to 2.5 millimoles per litre. This solution diluted fifty times was used for growing the roots. Variations in the absorption solution will be described in another section of this paper.

As the roots were excised, it was thought that during the course of the potassium absorption period, they might become deficient in the carbohydrate necessary to produce the energy required for active absorption of potassium. To offset this possible limiting factor, enough sucrose was added to the absorption solution to give a concentration of 2% of this compound.

The absorption of potassium was measured by suspending 2 g of excised roots in 40 ml solution in a 150-ml Erlenmeyer flask in a water bath at 26°C and aerating with oxygen. A bank of 10 such flasks was set up and one removed every 30 minutes and the concentration of potassium in the solution was measured flamephotometrically. The decrease in concentration gave the amount absorbed by the roots. To prevent loss by evaporation due to the aeration, the oxygen was passed through several bottles of distilled water before being bubbled through the solutions. To ensure uniformity of aeration in each flask, they were also connected at the outlet side to gas bubblers and the rate at which the gas bubbled through each was carefully regulated so that all flasks were aerated at the same rate.

RESULTS

The results recorded here show the effect of pH from 3.5 to 7.0 on the rate of absorption of potassium. As described above, the pH was adjusted by the addition of alkali before starting each experiment. It is seen (Fig. 1) that the rate of absorption rises as the

initial pH of the absorption solution is raised. The rate of uptake tends to decrease slightly with time. This was explained by the fact that in the absorption solution used, the pH invariably dropped to about 3.9 (Fig. 2) over the course of the experiment *i.e.*, in about 5 hours. It was seen that between pH 6.0 and 7.0 there is very little difference in the rate of uptake of potassium. It will also be seen that at the lowest pH there is an initial secretion of potassium (recorded as a negative absorption) which is reversed when the pH

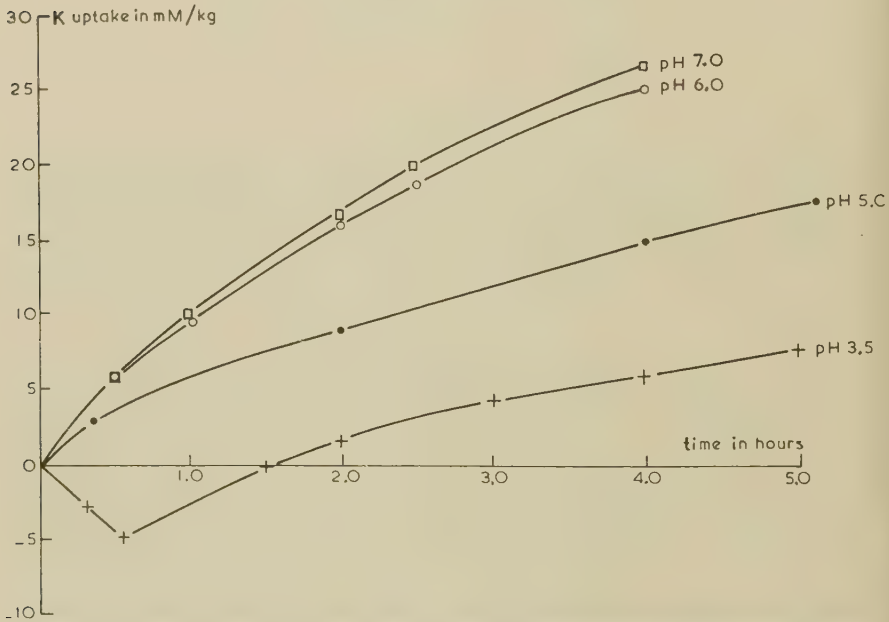

Fig. 1. Graph showing increase in the rate of potassium absorption as initial pH of culture solution is raised from 3.5 to 7.0.

of the absorption solution rises above 3.8 as is shown in Fig. 2. Unrecorded experiments have shown that below pH 3.0 the roots lose potassium to the external solution and that they ultimately lose the power to absorb potassium at higher pH values. This is in agreement with the work of various other authors [1 2 5].

Examination of these results shows that the rate of absorption during the first 30 minutes is only slightly influenced by changes in pH between 4.0 and 7.0. This is interpreted as meaning that the initial apparent absorption is mostly due to passive diffusion of

potassium into to the free space in the roots. That it is not completely due to this is shown by the temporary loss of potassium at and below pH 3.5 and to the slight decrease in absorption rate in the first 30 minutes at pH 5.0.

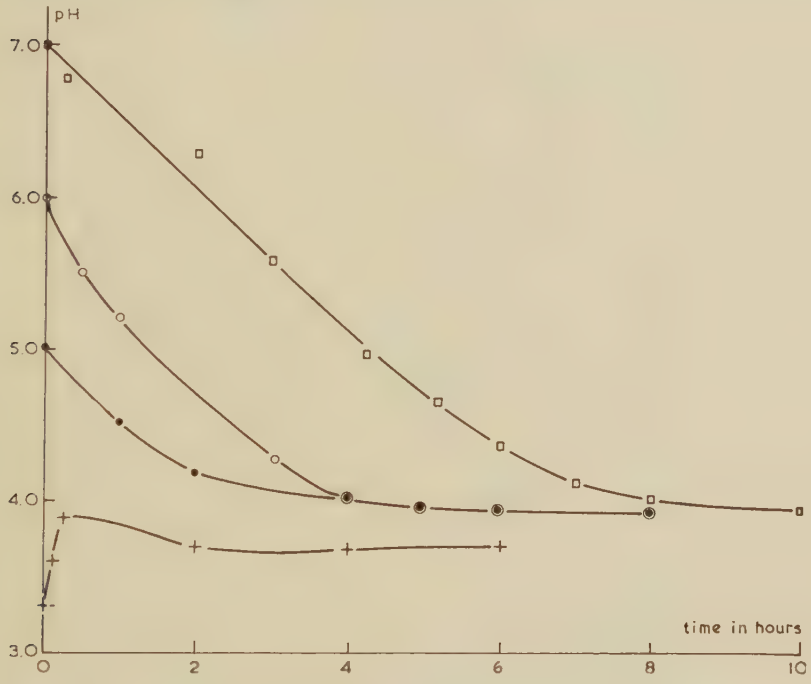

Fig. 2. Rate of fall of pH during K^+-uptake varying the initial pH in slightly buffered solution containing sucrose.

As described earlier, the absorption solution used in the above experiments contained 2 per cent sucrose as a source of carbohydrate. In view of the fact that there was such an unusual decrease in the pH in the above experiments, it was decided to repeat some of these experiments in the absence of sucrose. The potassium uptake was examined at pH 6.0 and pH 5.0, and it was seen (Fig. 3) that the presence of sucrose had no marked depressant effect on the rate of potassium absorption over an extended period. It was also seen that in the absence of sucrose, the pH decreased by only one unit over the time of the experiment.

Because of this sucrose effect, the uptake of sucrose was measured.

This was done colorimetrically, using the anthrone reagent [4]. This was done by suspending 20 g of excised roots in 80 ml of absorption solution containing varying quantities of sucrose and removing 1 ml at hourly intervals for analysis. This was diluted 25 times with distilled water and the sucrose content estimated. The results

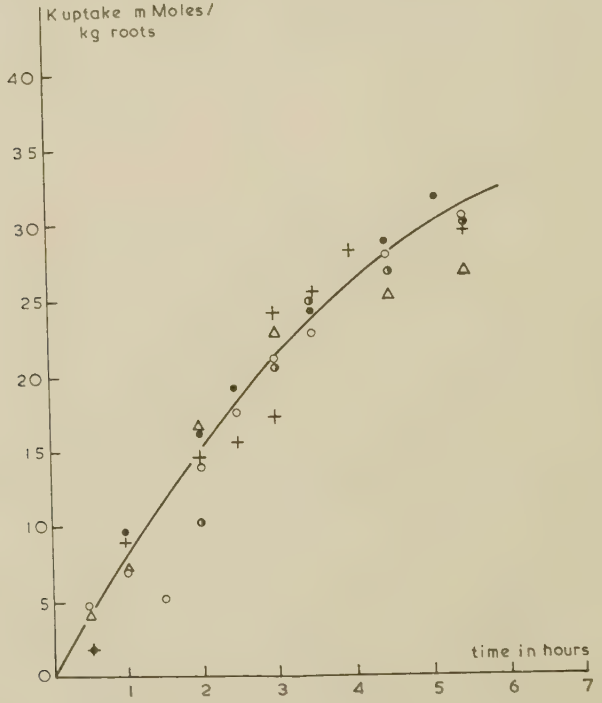

Fig. 3. Effect of varying concentration of sucrose on K^+-uptake by excised roots.

Initial pH of culture solution = 6.0

● = 1.0 m*M* sucrose ◑ = 3.0 m*M* sucrose
○ = 2.0 m*M* sucrose △ = 4.0 m*M* sucrose
\+ = 0.0 m*M* sucrose

given in Fig. 4. show that the sucrose is absorbed by the plant roots and that the rate of absorption is governed by the concentration of this compound below a sucrose concentration of 3.0 millimoles per litre, but that above this figure the absorption does not increase as the sucrose content is raised above this level. It is evident from these data that some factor imposes a limit on the rate

of sucrose absorption. This may be either the number of absorption sites on the cell membrane or the uptake transport mechanism. No attempt has so far been made to decide which of these is the limiting factor. That there is a definite relationship between

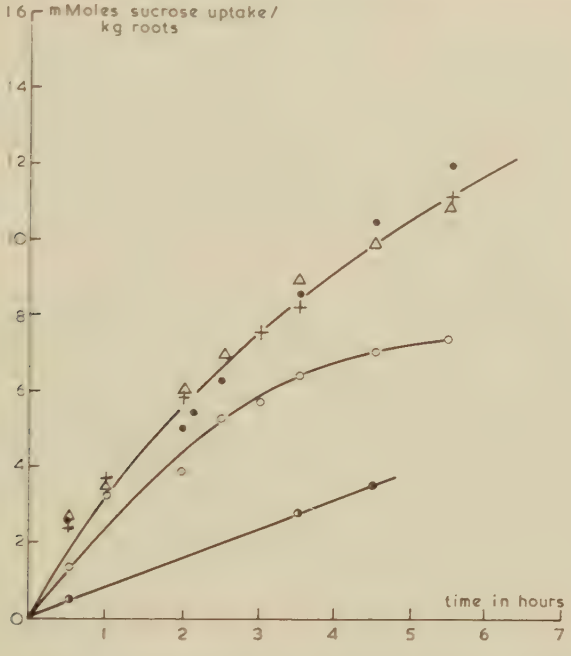

Fig. 4. Sucrose uptake in varying concentrations of sucrose
◑ = 1.0 m*M* sucrose
○ = 2.0 m*M* sucrose
△ = 3.0 m*M* sucrose
\+ = 4.0 m*M* sucrose
● = 5.0 m*M* sucrose

sucrose absorbed and hydrogen ion secreted can be seen from Fig. 6, which shows that for every molecule of sucrose absorbed two hydrogen ions are liberated. As this hydrogen-ion liberation is not related to the potassium absorption, it is assumed that the hydrogen ions are not liberated by the same mechanism that has been suggested for cation uptake, but are probably the result of some reaction occurring at the sucrose binding sites.

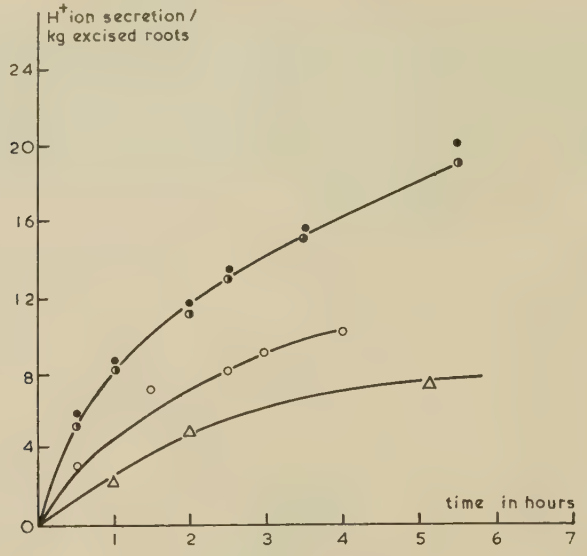

Fig. 5. H^+-ion secretion into culture solution by excised roots in varying concentrations of sucrose in the culture solution.

△ = 1.0 m*M* sucrose ◑ = 3.0 m*M* sucrose
◯ = 2.0 m*M* sucrose ● = 4.0 m*M* sucrose

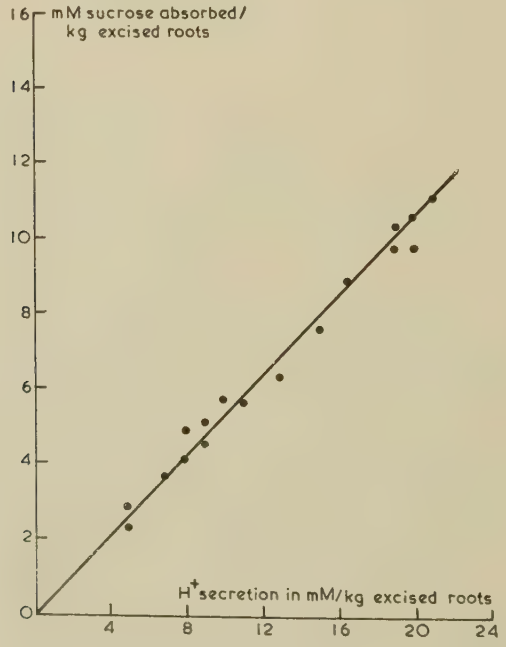

Fig. 6. Relationship of H^+-secretion to sucrose uptake.

CONCLUSIONS

It has been shown that the hydrogen-ion concentration in the external medium affects potassium absorption in the same way as would be expected from the equation

$$K^+ + HR \leftrightarrows KR + H^+$$

i.e. the potassium uptake is diminished in increasing concentrations of hydrogen ion. It has been shown that at pH 3.5 the reaction is driven in the reverse direction liberating potassium and that at slightly higher pH values, the potassium uptake is as would be expected, relatively slow. This is in agreement with similar work done on barley roots by Fawzy *et al.*[1].

It has also been shown that in the presence of sucrose there is a large secretion of hydrogen ions, but that the sucrose concentration does not affect the potassium uptake, except to decrease the uptake rate slightly as the external pH falls. It is therefore assumed that the potassium uptake is not directly related to the carbohydrate metabolism. The uptake of sucrose has been investigated and it was seen that the rate of uptake increases with a limited increase in the sucrose concentration showing that sucrose does not diffuse passively into the cells but is absorbed by some metabolic reaction which is saturated at a definite sucrose concentration in the absorption solution.

ACKNOWLEDGEMENTS

I wish to thank my assistant, Mr. S. Molloy, who did most of the analytical work involved in the above experiments. I would also like to thank Dr. T. Walsh of the Department of Agriculture for his advice in compiling this paper.

Received June 16, 1958

REFERENCES

1 Fawzy, H., Overstreet, R., and Jacobson, L., The influence of hydrogen ion concentration on cation absorption by barley roots. Plant Physiol. **29**, 234–237 (1954).

2 Hand, R. G., and Sutcliffe, J. F., Effect of pH on salt uptake by plants. Nature (London) **180**, 233 (1957).

3 Jacobson, L., Overstreet, R., Hang, H. M., and Handley, R., A study of potassium absorption by barley roots. Plant Physiol. **25**, 639–647 (1950).

4 Murphy, R. P., A method for the extraction of plant samples and the determination of total soluble carbohydrates. J. Sci. Food Agr. (In press).

5 Nielson, T. R. and Overstreet, R., A study of the role of the hydrogen ion in the mechanism of potassium absorption by excised barley roots. Plant Physiol. **30**, 303–309 (1955).

Plant and Soil X, no. 3 March 1959

THE INFLUENCE OF NUTRITION ON THE NITROGENOUS CONSTITUENTS OF PLANTS

I. NITROGEN AND PHOSPHORUS POT EXPERIMENTS WITH OATS

by CHRESTEN SØRENSEN

Department of Soil Fertility and Plant Nutrition,
The Royal Veterinary and Agricultural College, Copenhagen

Nitrogen fertilization usually leads to a higher yield of dry matter, and of grain; the yield of the latter as percentage of the total yield, however, falls. The response to phosphorus nutrition is generally a small increase in the dry-matter yield.

Information as to the influence of the nutrients in question on the content of the various nitrogen fractions during the period of growth is less readily obtainable. The present experiment was carried out in order to obtain information on this problem and on the variation in certain morphological characteristics.

METHODS

The experiment was carried out in cylindrical zinc pots coated on the inside with paraffin wax. The pots contained 13.5 kg of a sandy cultivated heath soil and had a surface area of 134 sq. cm. The soil contained 5.9 per cent humus and 0.01 per cent $CaCO_3$. 93.5 per cent of the soil had a particle size of less than 2 mm. Further soil analysis gave the following: —pH 6.2; 192 ppm P (10 g of soil extracted with 250 ml 0.1 *M* H_2SO_4 for 2 h); 66 ppm K (10 g of soil extracted with 100 ml 0.5 *M* NH_4Ac overnight); 4.9 ppm Cu (50 g of soil extracted with 200 ml 20 per cent HCl for 1 h), and 1.3 ppm Mn (10 g of soil extracted with 50 ml 1 *M* $Mg(NO_3)_2$ for 2 h).

From the results of the soil analysis it may be assumed that of the nutrients tested only a deficiency of manganese and a shortage of potassium would be expected at high nitrogen dressings.

The nutrient status of the soil, at the conclusion of the experiment is summarized in Table VII.

1. *Design of the experiment*

The following treatments were applied

N_0P_0	N_0P_1	N_0P_2
N_1P_0	N_1P_1	N_1P_2
N_2P_0	N_2P_1	N_2P_2

where

N_0 = no N	P_0 = no P
N_1 = 0.8 g N per pot	P_1 = 0.36 g P per pot
N_2 = 2.0 g N per pot	P_2 = 0.72 g P per pot

Nitrogen was supplied as $Ca(NO_3)_2$ and phosphorus as H_3PO_4. As a basic dressing, each pot in all treatments was supplied with 0.98 g K, 0.13 g Cu, 0.18 g Mn, and 1.0 g Mg as sulphate, and 40 mg B as borax. 11 replications were made. The soil was kept at a moisture content equal to 60 per cent of its total water-holding capacity calculated on an air-dry basis.

2. *Notes on growth*

On 21/4, 1954, 25 oat seeds (variety: Stål) were sown in each pot. Germination occurred on 5/5. On 24/5 the plants were thinned to 16 plants per pot. On 31/5 the plants showed satisfactory development and clear differences in growth were to be observed between treatments. In some plants the leaf apices were yellowish – as in the case of potassium deficiency, and on 4/6 each pot was supplied with a further 1 g K. Earing continued from 22/6 to 30/6. On 30/6 the oats in the P- and N-fertilized pots showed symptoms of Mg-deficiency. Fig. 1 illustrates the development of the crop on 12/7.

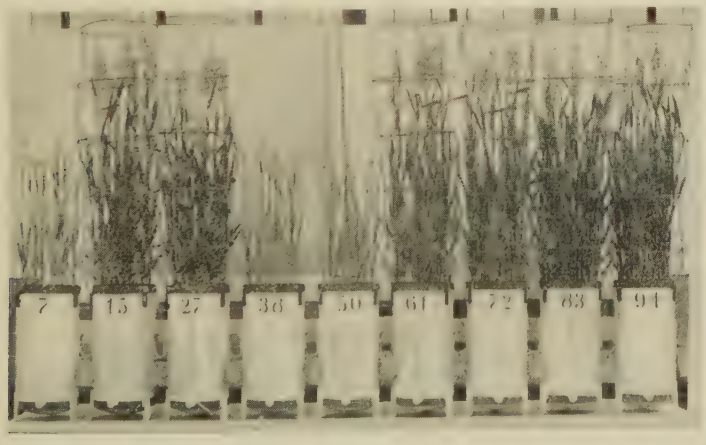

Fig. 1. Photo 12/7 of pots from the different treatments.
From left to right: N_0P_0, N_1P_0, N_2P_0, N_0P_1, N_0P_2, N_1P_1, N_1P_2, N_2P_1, N_2P_2.

On 22/6 and again on 12/7, 3 replications of each treatment were harvested. The remaining 5 replicates were harvested at maturity. On 18/8 the following

treatments were harvested: N_0P_0, N_1P_0, N_0P_1, N_0P_2, N_1P_1, and N_1P_2, and on 30/8, the N_2P_0, N_2P_1, and N_2P_2 treatments.

In order to check whether the symptoms observed on 31/5 and 30/6 were related to K- and Mg-deficiencies, celeriac was planted, or bean, clover or oats sown, in the pots from the harvest of 22/6, without any further addition of nutrients. Similarly, oats were sown in the pots from the harvest of 12/7. Of the three replicates from 12/7, one was supplied with 2 g K per pot, another with nitrogen equivalent to the amount absorbed by the preceding crop, and to the third, both K and N were added. In this subsequent experiment with oats following the harvest of 12/7, symptoms similar to potassium deficiency appeared again; but the yield of dry matter was the same whether the pots were supplied with potassium or not. None of these subsequent experiments confirmed the suspicion of K- and Mg-deficiencies in the main experiment, and the symptoms similar to those of K-deficiency are assumed to have been due to other factors, possibly cold climatic conditions.

3. *Harvesting*

At each harvest the crop was weighed immediately after cutting and the straw cut up into 1-cm pieces. Samples were taken for the determination of dry matter, and from the remaining fresh material about 75 g of each replicate were taken for the extraction of soluble nitrogen. Each harvest was made in the morning in cloudy weather. The time between harvesting of the crop and extraction was kept to a minimum of about 30 minutes for the harvests of 22/6 and 12/7; for the harvest at maturity, the corresponding time amounted to 2 to $2^1/_2$ hours.

4. *Extraction and concentration*

The fresh samples were wrapped in cheese cloth and dropped into 300 ml of boiling water over a strong flame; after 4 minutes the material was squeezed by hand, and dropped into another 300 ml of boiling water for 2 minutes. This procedure was repeated once more for 1 minute.

The fiber residues from the extraction can be assumed to contain the proteins as the main nitrogenous constituents; they were dried at 70°C, and their nitrogen contents, determined by Kjeldahl analysis, designated as protein nitrogen.

The extracts were collected, 20 ml toluene added, and stored at 4°C for a few days until they could be concentrated under vacuum on a water bath at 40°C. When the volume was reduced to about 40 ml the peptides were then precipitated by the addition of three volumes of ethanol. After centrifuging at 3000 r.p.m., the precipitate, which in addition to peptides contained some protein, was washed twice with 75 per cent (v/v) ethanol and the nitrogen content, determined by Kjeldahl analysis called peptide nitrogen. The supernatants were collected, concentrated under vacuum to about 30 ml, made to volume with water, 10 ml of toluene added, and stored at 4°C until analyzed. The total nitrogen in the supernatants is designated as soluble nitrogen.

5. *Nitrogen determinations*

Determination of total nitrogen was carried out by a micro-Kjeldahl technique, using a copper-selenium catalyst, digestion being continued for 5 hours after the samples had turned green. Before the determination of Kjeldahl-N in the straw from all harvests, in the spikelets from the second harvest, in the peptide precipitates and in the supernatants, reduction of nitrate was carried out: – 100 mg of dry matter was treated with 200 mg of ferrum reductum and 2 ml of diluted sulphuric acid (2 vol. H_2O : 1 vol. H_2SO_4), heated in a boiling water bath for 5 hours and set aside overnight.

Nitrate in the straw samples and in the spikelets from the second harvest, 12/7, was determined by the previously published modification of the xylenol method (Sørensen [7]).

6. *Determination of potassium and phosphorus*

Wet ashing was carried out using a mixture of sulphuric and perchloric acids. In the resulting solution, potassium was determined by a Beckman flame spectrophotometer, and phosphorus by the molybdenumblue method using stannous chloride.

RESULTS

Corresponding to the various treatments, a noticeable difference in the number of tillers was observed. The following data represent the means of 5 replicates for the number of tillers per 16 plants (per pot) on 5/6:

Treatment	N_0P_0	N_1P_0	N_2P_0	N_0P_1	N_0P_2	N_1P_1	N_1P_2	N_2P_1	N_2P_2
Number of tillers	0.2	25.2	14.2	0	0	28.0	26.8	17.2	21.0

Phosphorus alone did not increase the number of tillers but a considerable increase was found in the nitrogen-fertilized plants, and there seems to be a positive interaction between nitrogen and phosphorus. The number of tillers was less for N_2 than for N_1, a finding which is in agreement with the results reported by Pedersen [3].

Table I gives the amount of dry matter in the straw and in the spikelets at different times during the growing period.

As a "single" nutrient, phosphorus had little influence either on the percentage of dry matter or on the yield, but, when added with nitrogen, increased the yield of dry matter, the higher the nitrogen supply, the greater the increase.

For the harvest of 12/7, and even more so for the harvest of 22/6,

TABLE I

Percentage of dry matter and g of dry matter per pot at different times of the growing period

Treatment	Harvest 22/6		Harvest 12/7					Harvest 18/8: N_0P_0, N_1P_0, N_0P_1, N_0P_2, N_1P_1, and N_1P_2. Harvest 30/8: N_2P_0, N_2P_1, and N_2P_2				
	Whole plant		Straw		Spikelets		Total	Straw		Spikelets		Total
	%	g	%	g	%	g	g	%	g	%	g	g
N_0P_0	20.5	6.7	24.8	8.6	36.7	2.6	11.2	32.0	6.1	75.0	6.8	12.9
N_1P_0	17.8	29.4	24.2	47.2	36.4	14.8	62.0	32.5	33.8	76.8	38.9	72.7
N_2P_0	15.9	21.4	20.0	40.1	34.2	14.0	54.1	36.3	35.4	78.0	44.8	80.2
N_0P_1	20.0	6.6	24.8	8.4	37.1	2.7	11.0	32.1	6.0	74.5	6.2	12.2
N_0P_2	20.2	6.4	25.3	8.5	37.0	2.7	11.3	31.6	6.0	76.1	6.7	12.8
N_1P_1	17.6	32.3	23.9	47.8	36.9	14.4	62.2	31.1	34.5	76.2	40.8	75.4
N_1P_2	17.6	32.5	25.2	49.2	36.2	14.9	64.1	32.0	35.7	76.5	42.0	77.7
N_2P_1	15.6	26.5	19.2	48.1	33.4	15.1	63.2	44.7	41.8	80.0	53.1	94.9
N_2P_2	15.0	26.3	18.5	49.6	32.0	14.9	64.5	41.6	44.6	79.7	56.0	100.5

increasing the nitrogen level from N_1 to N_2 had a depressing effect on the yield of dry matter, and corresponding with this, a high nitrate content was found in the N_2-fertilized crops (cf Table V). For the harvests on 18/8 and 30/8, the yield increased from the N_1 to N_2 levels of fertilizer.

The data in Table II show the influence of nutrition and stage of development on the structure of the yield.

TABLE II

Structure of the yield at different times of the growing period (All data are calculated on dry matter)

Treatment	Harvest 12/7	Harvest 18/8 and 30/8					
	Spikelets as per cent of total yield	Spikelets as per cent of total yield	Chaff as per cent of spikelets	Seeds as per cent of total yield	Number of seeds per pot	Wt. of 1000 seeds g	Number of seeds per straw
N_0P_0	23.2	52.5	8.0	48.4	195	32.0	12.0
N_1P_0	23.9	53.6	6.1	50.4	1090	33.6	26.5
N_2P_0	25.9	55.6	6.0	52.5	1372	30.7	45.7
N_0P_1	24.5	51.1	7.4	47.1	195	29.5	12.2
N_0P_2	23.9	52.9	7.4	48.9	208	30.0	13.0
N_1P_1	23.2	54.2	6.1	52.0	1154	33.3	26.2
N_1P_2	23.2	54.2	6.0	50.8	1142	34.6	26.7
N_2P_1	23.9	56.1	6.4	52.4	1738	28.6	52.4
N_2P_2	23.1	56.0	6.2	52.2	1812	29.0	49.0

As a percentage of the total yield, the spikelets show little variation with nutritional treatment but this has an appreciable influence on the relative proportions of kernels and chaff in the spikelets. The seed weight decreases with heavy applications of nitrogen whilst the number of seeds per stalk increases with increasing nitrogen fertilization, in agreement with the results reported by Pedersen [3].

Increasing the phosphorus supply may have increased the rate of development.

During the period from 12 7 to maturity, the relative increase in dry-matter production of the N_2P_0- and N_2-plus-P-fertilized crops was very much greater than that of the other treatments: This suggests that the oat plants are possibly able to utilize appreciable amounts of nitrogen late in the growing period. The figures given in the last column of Table III show that the relative increase per day is also greater for these treatments than for others, probably due to a younger physiological state of the plants receiving high nitrogen supplies.

TABLE III

Increase in the yield of dry matter at different periods during the growing season

Treatment	g dry matter per pot 22/6	Increase per pot from 22/6–12/7			Increase per pot from 12/7 until maturity		
		g dry matter	%	Average daily per-centage	g dry matter	%	Average daily per-centage
N_0P_0	6.7	4.5	67	3.4	1.7	15	0.41
N_1P_0	29.4	32.6	111	5.6	10.7	17	0.46
N_2P_0	21.4	32.7	152	7.6	26.1	48	0.98
N_0P_1	6.6	4.4	67	3.4	1.2	11	0.30
N_0P_2	6.4	4.9	77	3.9	1.5	13	0.35
N_1P_1	32.3	30.1	93	4.7	13.2	21	0.57
N_1P_2	32.5	31.6	97	4.9	13.6	21	0.57
N_2P_1	26.5	36.7	138	6.9	31.7	50	1.02
N_2P_2	26.3	38.2	145	7.3	36.0	56	1.15

The treatments also influenced the amount of dry matter which could be extracted by boiling water. At maturity, 6 to 8 per cent was extracted from the non-nitrogen-fertilized plants, 10 to 14 per cent from the N_1-fertilized, and 14 to 18 per cent from the N_2P_0- and N_2-plus-P-fertilized plants.

PLANT ANALYSES

Some of the results obtained from the plant analyses are represented graphically in Fig. 3.

Two types of yield curve may be drawn – that in which the yield (ordinate, y) is plotted against the amount of nutrient supplied (abscissa, x_1) and, if the content of the nutrient in question has been determined in the dry matter, that in which the yield is plotted against the amount of nutrient absorbed by the plant (abscissa, x).

The two curves are related by the equation $x = a + b.x_1$, where a is the amount of nutrient absorbed by the unfertilized crop and b is the coefficient of absorption, which varies with the corresponding amount added (x_1) of the nutrient in question.

As pointed out by Steenbjerg [6], the second way of drawing the yield curve has two advantages – (1) the point of origin of the yield curve may, in practice coincide with the zero point of intersection of the x- and y-axes (because without absorption of nutrient, no yield would be produced), and therefore is useful for extrapolating from experimental data and (2), the s-shaped yield curve, often difficult to observe in the (x_1,y)-system, may often appear in the (x,y)-system. The reason for this is that the coefficient of absorption (b) usually falls with increasing amounts of nutrient added.

Steenbjerg [6] mentions that s-shaped curves have often been observed in well-performed experiments with all the common nutrients.

A curve of the latter form is presented in Fig. 2 (curve c), where x is the amount of nutrient absorbed.

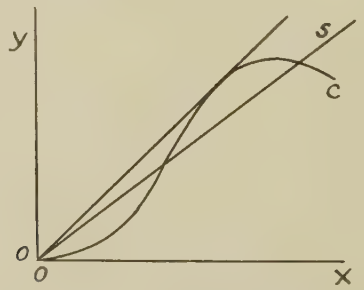

Fig. 2. Diagrammatic yield curve.

In this figure, s represents the secant to the curve c; as s rotates about the origin (0.0), it's coefficient of slope (α), at any point of

intersection with c, will be a linear function of the reciprocal of the percentage of the added nutrient in the dry matter ($1/a \cdot 100$ = per cent of nutrient in dry matter). From this it is easily seen that the percentage of nutrient in the dry matter will decrease as the yield increases up to the point where s forms the upper tangent to curve c; on either side of this point the percentage of nutrient increases with both increasing and decreasing yield. Hence the point on c, at which s forms the tangent, corresponds to the points of minima of the curves in Fig. 3a–3f, and *vice versa*. The points in Figures 3a–3f have been connected by smooth curves in agreement with the previous discussion and in the same sequence as the points on the corresponding yield curves.

From the Figures 3a, 3b, 3d, and 3e it is seen that the percentage of potassium follows a pattern very similar to that of the percentage of nitrogen. For the first and second harvests, the content of both nutrients increases even with the larger amount of nitrogen added to the growth medium. These curves are in agreement with the results reported by Müller [2] and Russel *et al.* [4].

It is possible that a high content of potassium is attained to maintain the ionic balance in the plant (cf the high content of nitrate, Table V), and from the comparison of Fig. 3e with Fig. 3b it is seen that the percentage of potassium following nitrogen fertilization tends to increase more in the absence of added phosphorus than at the P_2-level. This effect of nitrogen on potassium uptake was also found by Smith and Wallace [5], and is probably a result of an increase in the root cation-exchange capacity. For the harvest at maturity the potassium curves do not follow the percentages of nitrogen so well, because of the back translocation of potassium at that stage.

From the Figures 3c and 3f it is seen that when phosphorus is not supplied, the percentage of this nutrient decreases with increasing amounts of nitrogen added to the pots; at the P_2-level, however, the percentage of phosphorus increases slightly from N_1 to N_2. In experiments with grasses, Lewis and Lang [1] and Russel *et al.* [4] also observed this effect of nitrogen fertilization. A possible reason is that the high phosphorus level has in some way maintained the ionic balance in the plants, and, as seen from the Figures 3g and 3j, the amounts of nitrogen absorbed are higher at the P_2-level than where no phosphorus is added. This phosphorus effect on

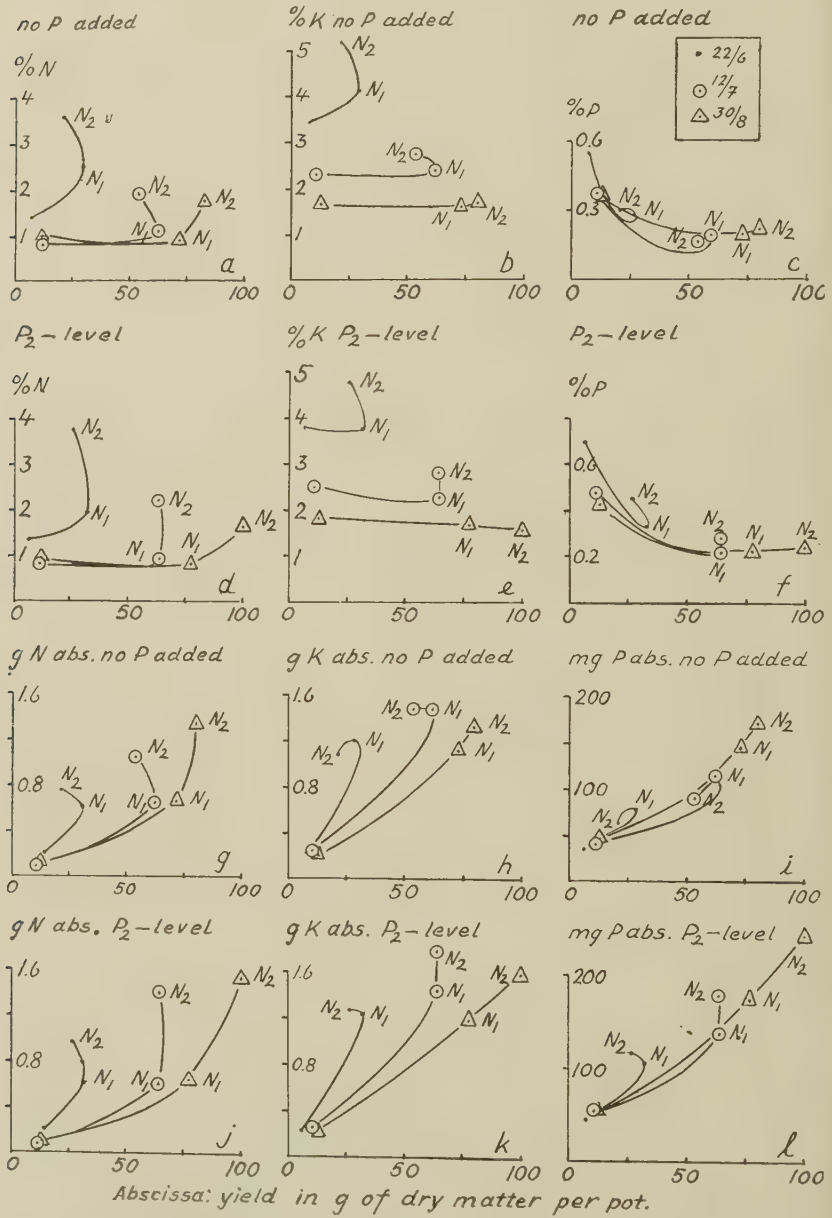
no P added
%N
%K no P added
no P added
• 22/6
⊙ 13/7
△ 30/8
%P
P2 – level
%K P2 – level
P2 – level
g N abs. no P added
g K abs. no P added
mg P abs. no P added
g N abs. P2 – level
g K abs. P2 – level
mg P abs. P2 – level
Abscissa: yield in g of dry matter per pot.

Fig. 3. In the figures a–f, the percentages of N, K and P, and in the figures g–l the absolute contents of N, K and P in the whole plant are plotted against g of dry matter produced per pot. The curves relate both to time of harvest and level of fertilization.

nitrogen absorption would possibly have been more pronounced if the content of available phosphorus in the soil itself had been lower. The percentage of phosphorus in the straw decreased during the growing period, and the decrease was most pronounced in the nitrogen-fertilized crops. Besides the greater growth increment in the latter the highest kernel percentage was also found in these crops.

Both on an absolute basis as well as relative to the total phosphorus content of the crop the translocation of phosphorus from the straw to the seed was greatest in the crops making the greatest amount of growth. Where phosphorus was added translocation increased with increasing nitrogen fertilization.

The amounts absorbed of the three nutrients in question are presented in the Figures 3g 3l. The amount of potassium absorbed depends on both the nitrogen- and P-fertilizer level. The amount of phosphorus absorbed follows a similar pattern, but while some return of potassium is noticeable during the period between the second and the final harvests, absorption of phosphorus has continued. This is probably due to the above-mentioned translocation.

When no phosphorus was added to the soil, the absorption of this nutrient by the first two harvests decreased from N_1 to N_2, this being mainly connected with the decrease in yield.

More detailed data on the absorption of nitrogen during the growing period are given in Table IV.

The relative content of nitrogen in the straw and in the crop as a

TABLE IV

Percentage and yield of total nitrogen at different times during the growing period
(The figures are calculated on dry weight, average per pot)

Harvest:	22/6		12/7					At maturity					
Treatment	Straw		Straw		Spikelets		Total	Straw		Seeds		Total	N in seeds as per cent of total N
	%	mg	%	mg	%	mg	mg	%	mg	%	mg	mg	
N_0P_0	1.36	91	0.74	63	1.24	32	96	0.39	30	1.56	97	127	77
N_1P_0	2.21	650	0.92	436	1.46	216	652	0.38	129	1.45	530	659	80
N_2P_0	3.61	773	2.00	803	1.82	255	1058	0.94	332	2.41	1014	1346	75
N_0P_1	1.25	83	0.71	59	1.23	33	92	0.38	23	1.53	88	111	80
N_0P_2	1.35	87	0.72	61	1.24	34	95	0.37	22	1.50	93	115	81
N_1P_1	2.09	673	0.88	419	1.49	215	634	0.34	118	1.41	542	660	82
N_1P_2	1.95	633	0.79	389	1.52	226	615	0.33	117	1.35	532	649	82
N_2P_1	3.57	945	2.34	1125	1.91	288	1413	0.81	338	2.45	1219	1557	78
N_2P_2	3.77	990	2.34	1162	1.94	289	1414	0.80	356	2.42	1271	1627	78

whole, decreases during the growing period, but at maturity the nitrogen content of the seeds is greater than that of the spikelets at the harvest of 12/7. The relative content of nitrogen is less dependent on the phosphorus nutrition than is potassium (cf Fig. 3); it increases with increasing nitrogen supply and is higher in spikelets and seeds than in straw, except for the N_2-fertilized crop harvested on 12/7. The amount of nitrogen in the seeds, as a percentage of total nitrogen in the crop, increases with phosphorus and nitrogen nutrition, but is smaller in the N_2- than in the N_1-fertilized plants. The reason for this may be that the production of dry matter in the N_2-fertilized plants had not ceased by 30/8; moreover, the seed weight was less in these plants (cf Table II).

If the increase in yield of total nitrogen is calculated in relation to the development of the plants, in the same way as was the increase in yield of dry matter (Table III), it will be seen that the relative increase in the yield of nitrogen from 22/6 to 12/7 was considerable only for the plants at the N_2-level.

The relative increase from 12/7 up to maturity was also important except for the plants at the N_1-level. The reason for this might be that nitrogen absorption by the non-nitrogen-fertilized plants and the plants at the N_1-level was in equilibrium with the mineralisation of nitrogen in the soil: The relative absorption then became lower for the plants at the N_1-level, but comparatively high for the non-nitrogen-fertilized plants. At the N_2-level there was still a reserve from the large amount of nitrogen added to the growth medium.

Results for the fractions of extractable nitrogen are shown in Table V.

The content of peptide-N as a percentage of total nitrogen is similar for the first and the second harvests, but increases at maturity. The content of soluble N as a percentage of total-N decreases from the first to the second harvest and then increases again.

The percentage of soluble N (including NO_3-N) in the dry matter is considerably higher in the N_2-fertilized crops than in the others. But the content of nitrate nitrogen influences these figures to an important extent, and therefore the percentage of soluble non-nitrate nitrogen has been calculated. It is seen that during the growing period, the soluble non-nitrate nitrogen as a percentage of

TABLE V

The variation in percentage and yield of nitrogen fractions in straw during the growing period

Treatment	a Peptide-N			b Sol. N minus NO_3-N			c Nitrate-N				a+b+c as % of total N
	% of total N	% in dry matter	mg per pot	% of total N	% in dry matter	mg per pot	% of total N	% of sol. N	‰ in dry matter	mg per pot	
Harvest 22/6											
N_0P_0	4.2	0.057	3.8	12.1	0.17	11.0	0.3	2.6	0.044	0.29	16.6
N_1P_0	2.6	0.058	17.0	11.3	0.25	73.0	2.0	15.3	0.45	13.2	15.9
N_2P_0	2.5	0.090	19.3	6.1	0.22	47.0	16.5	73.3	5.96	127.9	25.1
N_0P_1	4.0	0.050	3.3	13.9	0.18	11.4	0.2	1.5	0.026	0.17	18.1
N_0P_2	4.0	0.054	3.5	13.2	0.18	11.5	0.2	1.5	0.026	0.17	17.4
N_1P_1	2.6	0.055	17.6	11.2	0.22	75.3	0.1	0.6	0.014	0.45	13.9
N_1P_2	3.0	0.058	18.7	11.4	0.22	72.0	0.8	6.7	0.16	5.20	15.2
N_2P_1	1.8	0.065	17.1	4.3	0.15	40.7	18.1	81.0	6.45	170.7	24.2
N_2P_2	1.9	0.072	21.0	5.7	0.34	81.6	17.7	68.2	6.67	175.2	25.3
Harvest 12/7											
N_0P_0	3.2	0.024	2.0	9.2	0.07	5.7	0.4	4.9	0.03	0.26	12.8
N_1P_0	2.1	0.019	9.1	8.2	0.08	35.6	0.2	2.6	0.02	0.94	10.5
N_2P_0	2.3	0.046	18.5	7.4	0.15	59.3	14.1	65.3	2.83	113.5	23.8
N_0P_1	4.0	0.028	2.3	10.1	0.07	6.0	0.3	3.2	0.02	0.20	14.4
N_0P_2	3.3	0.023	2.0	9.2	0.07	5.6	0.9	8.5	0.06	0.52	13.4
N_1P_1	3.0	0.026	12.6	9.5	0.09	39.7	0.2	2.5	0.02	1.00	12.7
N_1P_2	2.9	0.023	11.4	10.6	0.09	41.3	0.3	2.7	0.02	1.13	13.8
N_2P_1	1.9	0.044	21.1	7.2	0.17	80.3	13.1	64.8	3.07	147.7	22.2
N_2P_2	1.6	0.038	19.0	5.7	0.14	66.8	14.2	70.8	3.32	164.7	21.5
Harvest at maturity											
N_0P_0	6.1	0.030	1.8	8.1	0.05	2.5	0.8	8.9	0.04	0.24	15.0
N_1P_0	5.7	0.022	7.4	11.0	0.05	14.3	1.0	8.7	0.04	1.35	17.7
N_2P_0A	5.3	0.050	17.5	5.3	0.05	17.3	27.2	74.3	2.55	90.3	37.9
N_2P_0B	5.4	0.050	17.3								
N_0P_1	9.9	0.038	2.2	12.6	0.05	2.9	0.5	3.3	0.02	0.10	23.0
N_0P_2	8.7	0.032	1.9	13.2	0.05	2.9	0.7	5.2	0.03	0.16	22.6
N_1P_1	8.3	0.028	9.8	13.8	0.05	16.3	0.2	1.4	0.01	0.23	22.3
N_1P_2	5.9	0.019	6.8	10.5	0.03	12.2	2.8	21.2	0.09	3.28	19.2
N_2P_1A	4.6	0.037	15.5	5.4	0.05	18.3	21.4	79.5	1.73	72.3	31.6
N_2P_1B	4.9	0.040	16.5								
N_2P_2A	5.6	0.045	19.8	6.1	0.05	21.5	14.1	70.0	1.13	50.3	25.4
N_2P_2B	4.8	0.039	17.2								

total nitrogen does not vary as much as the other nitrogen fractions. However, the nitrogen fraction concerned varies with the nutrition of the plants and is smallest for the N_2-fertilized plants. The content of soluble non-nitrate nitrogen in the dry matter varies both with the nutrition and with the development of the plants and decreases

METHODS

The birch stands of the eastern section of the reserve vary greatly in age and nine sample plots were marked out to give a series of plots covering a representative range of ages, with particular emphasis on stands older than the thicket stage. Few young trees occur in this region of the reserve and only one young plot, six years old, was set up, the rest being between 24 and 55 years of age. All of the sample plots were carefully located to avoid obvious breaks in the canopy.

The sample plots were usually a quarter of an acre in size and up to 250 living trees, selected at random, were measured in each plot for total height, bole diameter at breast height, canopy depth and spread. The six-year old trees differed greatly in size but the trees of the older stands were not so variable since the less-vigorous trees gradually die out as the woodlands mature.

In each plot, two representative trees of *Betula verrucosa*, having height, girth and canopy characteristics close to the plot average, were selected for detailed examination. The eighteen trees were felled in August 1954 and cut into 6-m lengths, measured from the apex, before being wrapped in hessian and quickly transported to the laboratory. Each tree was divided into leaf, branch and bole material, samples of which were taken and oven dried to a constant weight at 80°C. The root systems of four trees from different plots were carefully extracted by hand digging and winching and the dry weight of all root material, down to 0.5 cm diameter, was determined. All weights given in this account are oven dry.

WOODLAND FORM

The birch at Holme Fen produces a large number of viable seeds and, with suitable conditions for regeneration, a high stocking density is quickly achieved (Table I). Tree growth is rapid on the rich fen peat and the tree crowns soon meet to give a close canopy beneath which new seedlings cannot survive. Within six years from colonisation the birch forms a dense thicket and the lower, shaded branches begin to die. The dead branches are pruned naturally and the lowermost parts of the tree stems become completely branch-free. Although the number of living branches arising from each bole tends to reach a constant value within the first twenty years, the living canopy continues to increase in depth and is raised higher above ground level.

Changes in tree form are closely associated with changes in stocking density. For example, in unusually heavily stocked stands the average bole diameter is less than would be expected for the

TABLE VI

The non-nitrate nitrogen content of the straw during the growing period, calculated as percentage of dry matter and as yield in mg per pot (total nitrogen less nitrate nitrogen in dry matter)

Harvest	22 6		12 7		At maturity	
Treatment	%	mg	%	mg	%	mg
N_0P_0	1.36	91	0.74	63	0.39	30
N_1P_0	2.17	637	0.92	435	0.38	128
N_2P_0	3.01	645	1.72	690	0.68	242
N_0P_1	1.25	82	0.70	59	0.38	23
N_0P_2	1.35	87	0.71	61	0.36	22
N_1P_1	2.08	673	0.88	418	0.34	118
N_1P_2	1.93	628	0.79	388	0.32	113
N_2P_1	2.93	775	1.88	977	0.64	266
N_2P_2	3.10	815	2.01	997	0.69	306

TABLE VII

Results of soil analysis at the beginning and end of the experiment. Soil samples for electric conductivity taken on 24 6 and for pH, phosphorus, and potassium, on 1 9

	E.C. *	pH	ppm P	ppm K
At the beginning of the experiment	—	6.2	192	66
Treatment				
N_0P_0	0.19	6.4	143	122
N_1P_0	0.25	6.6	133	99
N_2P_0	0.37	6.7	119	95
N_0P_1	0.24	6.3	171	135
N_0P_2	0.23	6.1	237	131
N_1P_1	0.29	6.4	178	95
N_1P_2	0.23	6.3	223	106
N_2P_1	0.30	6.6	161	86
N_2P_2	0.31	6.5	237	74

* Electric conductivity in millimhos per cm at 20°C of the supernatant when 25 g of soil was shaken in 225 ml H_2O for 1 h

in Table VII. The figures for conductivity are not particularly high. The pH tends to become higher with increasing calcium-nitrate fertilization.

The amount of available phosphorus decreased during the growing period, except in the case of the P_2-fertilized treatments. The content of available potassium has increased.

DISCUSSION

According to the above results it appears that tiller formation is not simply related to nitrogen or phosphorus nutrition, but among other factors, it depends on the relative amounts added of both of these nutrients.

When harvested about the time of earing the heaviest application of nitrogen showed a depressing effect on the yield of dry matter, but at maturity the yield increased with nitrogen fertilization. This shows that it is not always sufficient to determine the effect of a fertilizer by measuring the yield only once at the end of the growing period or in the middle of it. In the present experiment the results also show that the oat plants are probably able to utilize appreciable amounts of nitrogen late in the growing period. This point of view is supported by the results (Tables III and IV) showing that the increase in dry-matter production and nitrogen absorption during the last part of the growing period is of almost the same magnitude for the non-nitrogen and N_1-fertilized crops, and on both a relative and absolute basis smaller than that for the N_2-fertilized crops. If it is assumed that that part of the nitrogen added to the N_1-fertilized crops, which has not been taken up by the plants by 12/7, is fixed by the soil and that the mineralization of nitrogen is about the same for the different treatments, then this might provide some explanation for the rather equal nitrogen absorption and dry-matter production of the N_0- and N_1-fertilized crops during the period mentioned.

On 12/7 there may still have been an excess of nitrate in the N_2-fertilized pots, whereby the plants have been kept in a younger physiological state, and the growing period was prolonged. This supposition is supported by the fact that the percentage of nitrogen in the straw on 12/7 is higher than in the spikelets, whereas these figures are reversed for the other treatments.

The yield of nitrogen in the seeds and that of non-nitrate nitrogen in the straw have been improved not only by increasing nitrogen fertilization, but also by increasing phosphorus fertilization at the highest nitrogen level. These results may be of interest both from the fertilization and nutritional points of view.

When the plants were fertilized with heavy amounts of nitrogen the content of non-extractable nitrogen, as a percentage of total

nitrogen in the straw, obviously increased with increasing phosphorus fertilization. These findings might be explained by a higher phosphorus content and a resulting higher content of organic phosphorus compounds stimulating protein synthesis. This problem, however, has not been attacked in the present experiment.

SUMMARY

A pot experiment has been made with oats fertilized with increasing amounts of calcium nitrate, phosphorus, and various combinations of these. The yield of dry matter and the content of phosphorus, potassium, total nitrogen, soluble nitrogen, peptide nitrogen, and nitrate nitrogen were determined on three separate occasions during the growing period.

The number of tillers was increased to a certain extent by nitrogen and then decreased again with further applications. Phosphorus alone had no influence, but a positive interaction between phosphorus and nitrogen on the number of tillers was observed. Nitrogen increased the number of seeds per spikelet and the percentage of grain.

Except at maturity the content of potassium followed the content of total nitrogen, the percentage of which increased with increasing nitrogen supply. The content of nitrate nitrogen decreased during the growing period and at maturity was decreased by increasing phosphorus supply.

As a percentage of the total nitrogen, the peptide nitrogen and soluble non-nitrate nitrogen in the straw decreased with increasing nitrogen supply. Peptide nitrogen increased at maturity. When the phosphorus was added with a heavy dressing of calcium nitrate, the percentage and the yield of protein nitrogen seemed to increase.

Received February 17, 1958

REFERENCES

1 Lewis, R. D., and Lang, L., Effect of nitrogen and yield of forage of eight grasses grown in high altitude meadows of Wyoming. Agron. J. **49**, 332–35 (1957).

2 Müller, K., Quantitative papierchromatographische Untersuchungen: Über die Abhängigkeit der Zuckerbildung in Weidelgras, Spinat, Roggen, Gerste, Mais und Futterrüben von der Kalium- und Stickstoffdüngung unter besonderer Berücksichtigung des Kaliums. Diss. Göttingen, 102 pp. (1957).

3 Pedersen, A., The effect of applying nitrate of lime to cereal crops. Festskrift til Knut Vik, Oslo 1951, 18–37 (1957).

4 Russell, J. S., Bourg, C. W., and Rhoades, H. F., Effect of nitrogen fertilizer on the nitrogen, phosphorus, and cation content of bromegrass. Soil Sci. Soc. Am. Proc. **18**, 293–94 (1954).

5 Smith, R. L., and Wallace, A., Influence of nitrogen fertilization, cation concentration and root cation-exchange capacity on calcium and potassium uptake by plants. Soil Sci. **82**, 165–72 (1956).

6 Steenbjerg, F., Manuring, plant production and the chemical composition of the plant. Plant and Soil **V**, 226–42 (1954).

7 Sørensen, C., The xylenol method and determination of nitrate in beets. Physiol. Plantarum **9**, 304–20 (1956).

USE OF ORGANIC PHOSPHATE IN NUTRIENT CULTURE *

by S. K. MAJUMDER ** and STUART DUNN

Department of Botany, University of New Hampshire, Durham, New Hampshire, U.S.A.

INTRODUCTION

The problem of iron supply for plants growing in nutriculture has long been a difficult one, especially with high concentrations of phosphorus and high pH levels. Many forms of organic iron, including chelates, have been used in attempts to avoid this difficulty [4,5]. There is no report in the literature of the use of organic forms of phosphorus in solution culture. Spencer and Willhite [4], however, found that in soil applications a number of organic phosphates are equally as available to plants as inorganic ones. Dunn and Roberts [2] successfully used magnesium propyl phosphate as a source of magnesium for apple seedlings in nutriculture, but this was not the major source of phosphorus.

The present paper deals with the effects of several organic phosphates on the growth of corn (maize) in solution culture and their interactions with iron supplied as $FeCl_3$.

EXPERIMENTAL

The corn plants were grown and harvested in exactly the same way as previously described [3]. The basic composition of the nutrient solution is given in Table I. Any deviation from this table will be indicated below.

* Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 226.

** This report is based on portions of a thesis presented in partial fulfillment of the requirements for the Ph.D. degree at the University of New Hampshire.

TABLE I

The basic composition of the solution, per liter			
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]*	[illegible]

* The solution of $FeCl_3$ was freshly prepared immediately before use, since any stock solution of this compound would deteriorate in contact with air.

Experiment 1

In this experiment there were five treatments, each represented by 12 cultures jars with two plants in each jar. The source of iron for each of the treatments was Fe-EDTA (10 micromoles per liter). Treatment A, which had received potassium dihydrogen phosphate as the source of phosphorus, was considered the control. Plants in treatment B received sodium tetraphosphate (0.26 millimoles per liter) as the only source of phosphorus. Plants in treatment C, D and E, on the other hand, received as the sources of phosphorus ethyl ammonium phosphate, ethyl acid phosphate and n-propyl acid phosphate respectively. The concentration of each of these organic phosphates was 0.48 millimoles per liter of culture solution. The only source of potassium in treatments B, C, D and E was potassium chloride (0.2 millimoles per liter). Other constituents of the solutions in all the treatments were the same as listed in Table I. The range of pH of the culture solutions throughout was between 4.0 and 5.0. Results are shown in Table II.

The results show that plants receiving ethyl ammonium phosphate (treatment C) grew as well as those of the control (treatment A). There were no visible symptoms of mineral deficiency in either of the two treatments. The average dry weights of shoots in treatments B, D and E, however, were significantly less than those of treatments A and C. The performance of treatment B is in partial agreement with the results of Drumheller[1] who suggested that sodium tetraphosphate compared favorably with KH_2PO_4 as a source of phosphorus in nutrient solution and that it promoted better root growth (Table II). Plants in treatment D and E were stunted; and the leaf blades were pale yellow in colour while the

TABLE II

The effects of organic and inorganic phosphates on the growth of corn in solution culture

Treatments	Concentration of P millimoles/liter	Dry weight grams/plant		Standard error	
		Top	Root	Top	Root
A. Potassium dihydrogen phosphate (control)	0.06	1.63	0.63	0.086	0.047
B. Sodium tetraphosphate	0.07	1.00 **	0.63	0.072	0.038
C. Ethyl ammonium phosphate	0.06	1.62	0.60	0.085	0.033
D. Ethyl acid phosphate	0.14	0.49 **	0.41 **	0.060	0.022
E. *n*-Propyl acid phosphate	0.14	0.75 **	0.48 *	0.047	0.013

* Significantly less than the corresponding values of both treatments A and C at 0.05 level.

** Significantly less than the corresponding values of both treatments A and C at 0.01 level.

bases of the blades and the sheaths were purple. These visual symptoms are known to be due to phosphorus deficiency. Such symptoms occurred in treatments D and E in spite of the fact that the plants here received about twice as much phosphorus as the plants in the other treatments. This indicates that the phosphorus from ethyl acid phosphate and *n*-propyl acid phosphate was not totally available to plants growing in solution cultures under these conditions.

The effective use of ethyl ammonium phosphate in solution culture, as is evident from the normal growth of plants in treatment C, raises two relevant questions: (a) How does the phosphorus of this compound become available to plants and (b) Can this compound be used in solution culture of higher pH in which the source of iron is inorganic? The answer to the first question is beyond the scope of the present investigation. The following experiment was designed, however, to gain information pertaining to the second question.

Experiment 2

There were 5 treatments in this experiment. Each treatment consisted of 5 culture jars with 2 plants in each jar. Plants in treatment A – the control - received potassium dihydrogen phosphate and Fe-EDTA as sources of phosphorus and iron. In treatment B, ethyl ammonium phosphate and ferric chloride were used as sources of phosphorus and iron respectively. The chemical and physical properties of ethyl ammonium phosphate were described elsewhere [3]. In treatment C, the sources of both iron and phosphorus were inorganic in form as listed in Table I. Iron of any form was omitted from treatment D. Treatment E, on the other hand, was totally free from any trace of phosphorus. The only source of potassium in treatment B and E was potassium chloride (0.21 millimoles per liter). Other constituents of the solutions in all the treatments were the same as listed in Table I. The pH of the solutions in all treatments was maintained between 6.0 and 7.0 by adding minute amounts of HCl or NaOH to the solutions. Results are shown in Figure 1.

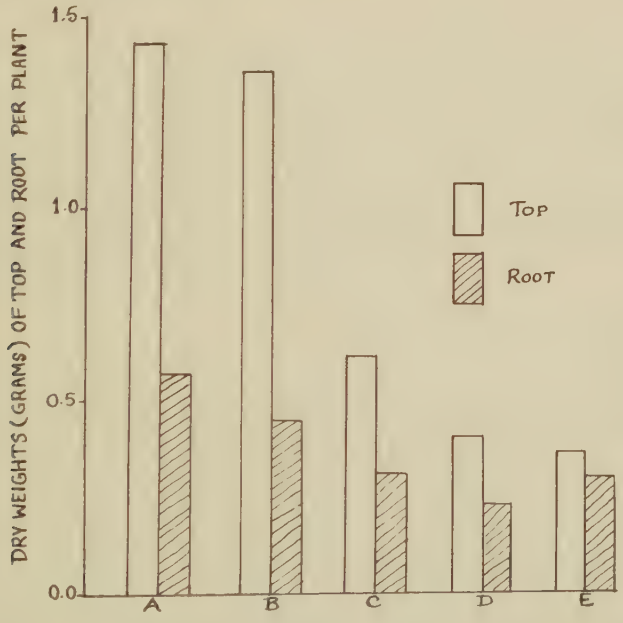

Fig. 1. Effect of iron-phosphate interaction on the growth of corn (maize) in culture solution.

A. KH_2PO_4 and Fe-EDTA; B. Ethyl ammonium phosphate and $FeCl_3$; C. KH_2PO_4 and $FeCl_3$; D. No Iron; E. No phosphorus.

The results indicate that the growth of plants in solution culture bears a distinct relation to the sources of iron and phosphorus. Plants in treatments A and B were equally vigorous in appearance and developed no visual symptoms of either iron or phosphorus deficiency (with treatments D and E as criteria of such deficiencies). Plants in treatment C, on the other hand, were very stunted and chlorotic in appearance which is indicative of iron deficiency.

The iron chlorosis in the plants of treatment C may be attributed to the fact that Fe^{+++} of $FeCl_3$ is progressively precipitated out by KH_2PO_4 at a high pH and becomes unavailable to the plants. This is in substantial agreement with previous workers. The results of the present experiment provide substantial evidence that the problem of iron supply at higher pH levels in nutrient culture can be overcome either by using chelated iron (Fe-EDTA) or by substituting ethyl ammonium phosphate for KH_2PO_4. This organic phosphate does not seem to interact with $FeCl_3$ in the same way as does KH_2PO_4.

SUMMARY

Ethyl ammonium phosphate proved to be a good substitute for KH_2PO_4 as a source of phosphorus for corn (maize) growing in nutrient solutions under greenhouse conditions. Ethyl acid phosphate and *n*-propyl acid phosphate were ineffective as sources of phosphorus under these conditions.

Ethyl ammonium phosphate does not seem to interact with $FeCl_3$ in the same way as does KH_2PO_4 at high pH (6.0–7.0) of the culture solution.

There appears to be no other published report of the successful use of organic phosphate as a source of phosphorus for plants growing in nutrient solution.

Received July 22, 1958

REFERENCES

1 Drumheller, P. Fern, Sodium tetra-phosphate as a source of phosphorus in mineral nutrition studies with plants. M.S. Thesis, University of New Hampshire, U.S.A. (1944).

2 Dunn, Stuart and Roberts, Suzanne S., The availability of magnesium from organic carriers in artificial substrates. Plant Physiol. **29**, 337–342 (1954).

3 Majumder, S. K., and Dunn, S., Effect of metal chelates on growth of corn in solution cultures. Plant Physiol. **33**, 166–169 (1958).

4 Spencer, V. E. and Willhite, F. M., Phosphate studies II: Chemical availability of phosphorus in various organic and inorganic carriers, as indicated by the Neubauer test. Soil Sci. **58**, 151–161 (1944).

5 Wallace, A., (ed.), Symposium on the use of metal chelates in plant nutrition. The National Press, Palo Alto, California (1956).

Plant and Soil X, no. 3 March 1959

THE GROWTH AND COMPOSITION OF NATURAL STANDS OF BIRCH

1. DRY-MATTER PRODUCTION

by J. D. OVINGTON * and H. A. I. MADGWICK

The Nature Conservancy, Merlewood Research Station, Grange-over-Sands, Lancashire

INTRODUCTION

Since natural regeneration is unreliable and difficult to control in the deforested landscape of Britain, it has rarely been used by British foresters in their efforts to build up a reserve of timber. It is not surprising therefore, that the production of stemwood by forest plantations has been studied more intensively than that of woodlands originating from natural regeneration. Large areas of open moorland have been planted with trees but, once woodland conditions are created in the new plantations, natural regeneration may prove to be of increasing importance for establishing the succeeding forest crops. This account is concerned with the productivity of natural stands of birch growing on deep, fen peat at Holme Fen Nature Reserve, near Peterborough.

Early in the nineteenth century Holme Fen was drained and reclaimed for agriculture. A few small coverts of mixed woodlands were planted in the area about 1870 and by the turn of the century the rest of the former fen had been invaded by birch and had reverted to woodland. The present mixed stands of *Betula verrucosa* and *B. pubescens* vary considerably in age, since some of the reserve has been cleared of timber at different times by accidental burning or wartime felling, and has been rapidly recolonised by birch. The Holme Fen woodlands have had little sylvicultural management and the stocking density is largely controlled by competition between trees. Many of the suppressed trees are attacked by *Polyporus betulinus* and decompose on the site without being harvested.

* Now at the Department of Botany, Minneapolis, Minnesota, U.S.A.

METHODS

The birch stands of the eastern section of the reserve vary greatly in age and nine sample plots were marked out to give a series of plots covering a representative range of ages, with particular emphasis on stands older than the thicket stage. Few young trees occur in this region of the reserve and only one young plot, six years old, was set up, the rest being between 24 and 55 years of age. All of the sample plots were carefully located to avoid obvious breaks in the canopy.

The sample plots were usually a quarter of an acre in size and up to 250 living trees, selected at random, were measured in each plot for total height, bole diameter at breast height, canopy depth and spread. The six-year old trees differed greatly in size but the trees of the older stands were not so variable since the less-vigorous trees gradually die out as the woodlands mature.

In each plot, two representative trees of *Betula verrucosa*, having height, girth and canopy characteristics close to the plot average, were selected for detailed examination. The eighteen trees were felled in August 1954 and cut into 6-m lengths, measured from the apex, before being wrapped in hessian and quickly transported to the laboratory. Each tree was divided into leaf, branch and bole material, samples of which were taken and oven dried to a constant weight at 80°C. The root systems of four trees from different plots were carefully extracted by hand digging and winching and the dry weight of all root material, down to 0.5 cm diameter, was determined. All weights given in this account are oven dry.

WOODLAND FORM

The birch at Holme Fen produces a large number of viable seeds and, with suitable conditions for regeneration, a high stocking density is quickly achieved (Table I). Tree growth is rapid on the rich fen peat and the tree crowns soon meet to give a close canopy beneath which new seedlings cannot survive. Within six years from colonisation the birch forms a dense thicket and the lower, shaded branches begin to die. The dead branches are pruned naturally and the lowermost parts of the tree stems become completely branch-free. Although the number of living branches arising from each bole tends to reach a constant value within the first twenty years, the living canopy continues to increase in depth and is raised higher above ground level.

Changes in tree form are closely associated with changes in stocking density. For example, in unusually heavily stocked stands the average bole diameter is less than would be expected for the

TABLE I

Average tree dimensions in birch plots								
Age	Ht.	No. of trees per ha	Ht. lowest living branch	Stem diameter at breast ht.	Min. canopy spread	Max. canopy spread	Ht. % max. canopy spread	*Betula verrucosa*
(years)	(m)		(m)	(cm)	(m)	(m)	(m)	(%)
6	2.1	10,450	0.8	—	0.2	0.5	1.5	96
24	9.1	4,990	5.5	6	0.3	1.1	7.8	72
27	12.8	2,480	8.1	10	0.4	1.6	11.0	77
32	9.5	4,210	5.7	7	0.4	1.3	7.8	84
38	12.3	1,500	6.8	12	0.6	1.9	11.0	84
42	13.0	1,340	7.4	14	0.7	2.2	10.3	88
46	18.8	740	10.7	21	0.6	2.7	15.4	89
53	18.5	1,020	10.6	18	0.6	2.6	15.2	87
55	17.6	880	9.3	19	0.7	2.9	14.2	88

tree age so that the individual bole weights are relatively low. As the woodlands mature, their stocking density is reduced and, although variations occur, the overall trend is for a 50 per cent mortality every ten years. The death of the smaller trees will automatically cause, not only a reduction in stocking density, but also an increase in the average size of the remaining trees. *Betula pubescens* is not so long lived as *B. verrucosa*, which consequently becomes the dominant species of the older woodlands.

SAMPLE TREES OF BIRCH

1. *Canopy*

(a) Branches. The total weight of branch material on the individual birch trees tends to increase with greater age, the increase being particularly marked throughout the pole stage (Table II). Up to about 40 years of age this increase can be attributed to greater amounts of both living and dead branches but, after 40 years, the weight of dead branches (*i.e.* branches not bearing green leaves) per tree remains relatively constant.

Counts were made of the number of living branches arising directly from the tree boles and the number becomes more or less constant, at about 50 per tree, from the pole stage onwards. Since the depth of living canopy tends to increase with age and the number of branches becomes constant, the average distance between branches is greater and the canopy is more open in the older

TABLE II

The number and weight of branches on sample trees					
Age (years)	No. of living branches	Av. wt. of living branches (g)	No. of dead branches	Av. wt. of dead branches (g)	Total wt. of all branches (kg)
6	29	1	10	1	< 0.1
24	49	48	24	23	2.9
27	59	60	1	290	3.9
32	46	49	8	84	2.9
38	61	119	25	96	9.7
42	44	154	8	418	10.1
46	54	423	6	443	25.5
53	50	601	2	1,235	32.5
55	49	626	4	488	32.6

woodlands. Some old branches arising low down on the tree stem remain alive and continue to enlarge as they keep pace with the upward growth of the crowns so that the average branch weight increases throughout the life of a tree.

(b) Leaves. Compared with branch material, the weight of leaves per tree is relatively small but increases as the trees mature (Table III). Even on the largest trees carrying approx. 76,000 leaves, the total weight of leaf material is less than 3 kg, and trees between 20 to 40 years old carry less than 0.5 kg of leaves. Some leaves may have been removed by being blown from the tree crowns

TABLE III

The average O.D. weight of sample trees									
Age (yrs.)	Wt. of leaves (kg)	Surface area of leaves (m^2)	No. of leaves (× 100)	Wt. of living branches (kg)	Wt. of living canopy (kg)	Wt. of bole (kg)	Wt. of living shoot (kg)	Wt. of roots > 0.5 cm diam. (kg)	Wt. of dead branches (kg)
6	< 0.1	0.3	4	0.1	0.1	0.1	0.1	< 0.1	< 0.1
24	0.5	12.4	114	2.4	2.9	9.6	12.5	3.4	0.5
27	0.5	14.2	155	3.6	4.1	27.5	31.6	—	0.3
32	0.4	9.8	132	2.2	2.6	12.6	15.2	—	0.7
38	0.4	11.6	196	7.3	7.7	39.3	47.0	—	2.4
42	0.8	20.8	257	6.8	7.6	43.7	51.3	19.3	3.3
46	2.1	55.4	694	22.8	24.9	138.3	163.2	—	2.7
53	2.2	58.3	681	30.0	32.2	137.0	169.2	—	2.5
55	2.8	74.1	756	30.7	33.5	152.9	186.4	56.5	1.9

or eaten by animals before the samples were taken but such losses did not appear to be of great significance at the time of felling.

Leaf area was determined by weighing groups of 100 circular leaf discs, of known area, cut from fresh leaves and subsequently dried. The leaf area values given in Table III are for both sides of the leaves and amount to about 75 sq. m. for the oldest tree.

2. *Bole*

Initially, the annual weight increment of the bole is small but becomes much greater after about twenty years. Bole weight and girth are greatly influenced by stocking density, thus the 27-year old stand is comparatively understocked and the average bole weight and girth in this plot are relatively large. The oldest trees at Holme Fen are approaching the maximum height for native birches and there is some evidence of a decrease in height growth and bole-weight increment after fifty years of age.

3. *Total shoot*

The relationships between the dry weights of the whole living shoot, inclusive of leaves, branches and boles, to the height and diameter of the boles have been determined for young trees of *Pinus sylvestris* (Rutter [7]). Similar relationships may be determined for the natural birch trees and the regression formulae are:

$\log_{10}W = -5.54 + 3.28 \log_{10}h$ S.E. of estimate = 0.11

$\log_{10}W = 1.54 + 2.60 \log_{10}d$ S.E. of estimate = 0.11

$\log_{10}W = -2.37 + 1.78 \log_{10}h + 1.25 \log_{10}d$ S.E. of estimate = 0.07

where W = shoot weight in grammes, h = tree height in centimetres and d = hypocotyl diameter in centimetres.

The correlation coefficients between log shoot weight and log height or log diameter are both over 0.99 so that there is relatively little advantage to be gained by combining the three factors in a multiple regression.

The proportions of leaf, branch and bole material change considerably with age. In the young seedlings and saplings, a large part of the dry matter is represented by the canopy but, as the trees grow, the canopy, and particularly the leaves, forms a smaller percentage of the total shoot weight. In the mature trees, the leaves represent only 1 to 2 per cent of the total shoot weight and the living branches about 18 per cent.

4. *Root system*

Only four root systems were sampled and they increase in weight as the trees become older. In general, the increase in root weight parallels shoot development and the living shoot to root ratio is fairly constant, being about 3 or 4. When the root systems were being excavated, numerous root fusions, between roots of the same tree and roots of different trees, were observed.

5. *Gross production*

As a tree grows, it does not retain all of the organic matter that it has produced during its lifetime, some material being lost as a result of respiration and the death of certain parts of the tree (Fig. 1). The loss of organic matter from a living tree by mortality does not occur equally for the different components of the tree, for example, the boles and branches are relatively long-lived compared with the leaves, which are shed after only a few months. Similarly the main lateral roots may persist throughout the life of a tree whilst some of the small, terminal rootlets may die annually.

Assuming that the sample trees form an age and developmental series, it has been possible to estimate, for individual trees, the gross production, at successive ages, of leaves, branches and bole but not of fruits and roots. The difference between gross production and the current weight of a tree continues to increase throughout its life and, after 55 years, the total production of leaves and branches is about 14 and 1.7 times the respective weights carried on the standing trees (Table IV).

TABLE IV

The total weight of dry matter produced by average sample trees				
After	Branches (kg)	Leaves (kg)	Bole (kg)	Whole shoot (kg)
6 yrs.	0.1	< 0.1	0.1	0.2
24 yrs.	7.2	4.4	9.6	21.2
27 yrs.	14.4	5.9	27.5	47.8
32 yrs.	8.0	8.2	12.5	28.7
38 yrs.	15.4	10.6	39.3	65.3
42 yrs.	22.1	13.1	43.7	78.9
46 yrs.	54.3	18.8	138.3	211.4
53 yrs.	48.5	33.9	137.0	219.4
55 yrs.	60.2	38.9	152.9	252.0

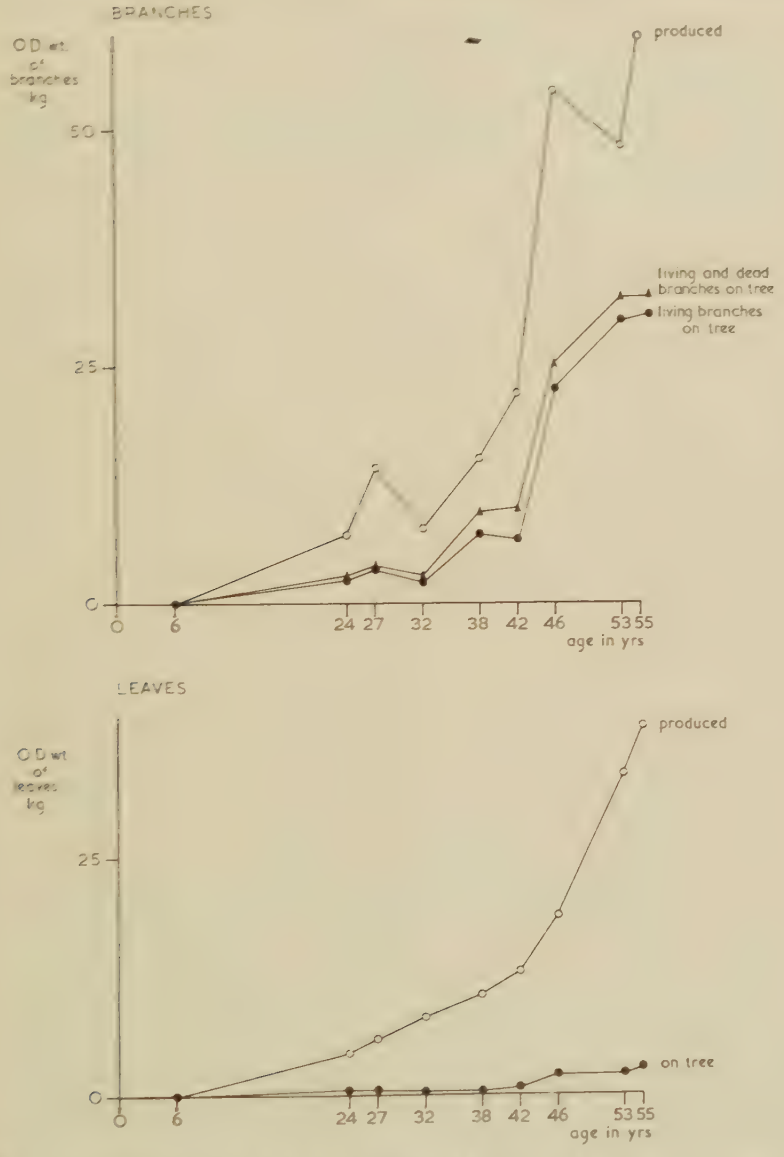

Fig. 1. Gross production and dry weight of leaves and branches of sample trees.

WOODLANDS OF BIRCH

1. *The standing crop*

The total amounts of dry matter contained in all the living trees of the natural birch stands were estimated by multiplying the average dry weights of the sample trees by the number of trees per hectare (Fig. 2).

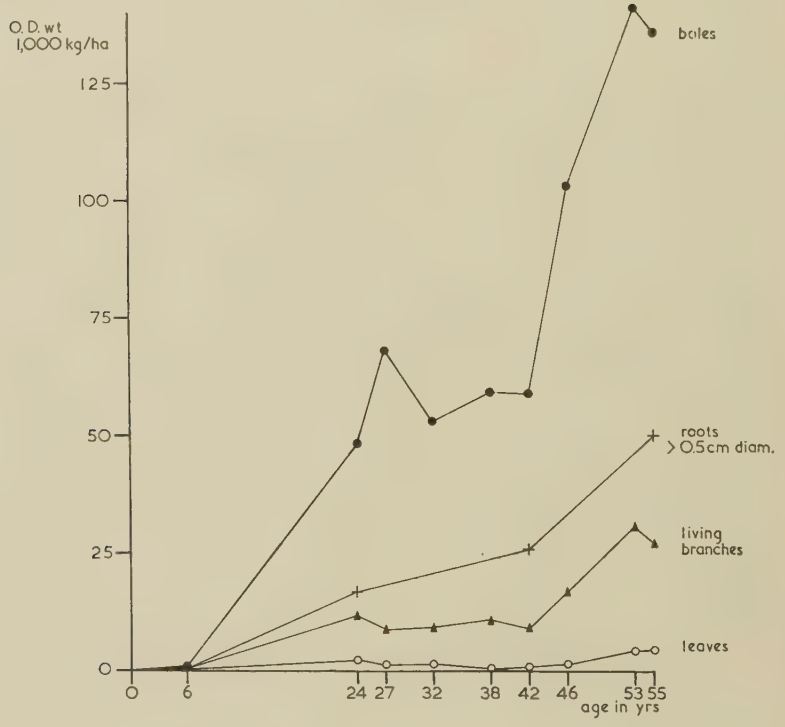

Fig. 2. The O.D. wt. of natural stands of birch.

During the first six years after colonisation, the weight of stem per hectare of woodland increases relatively slowly but afterwards, the increase in the total weight of the boles is more rapid (Table V). This rapid build up of bole material continues throughout the rest of the 55 years and is made possible by the high stocking density in the sampling stage.

The overall weight of living branches in each age class of woodland is much less than that of the boles but also increases as the stands mature. The continued increase in the total branch weight contrasts

TABLE V

The total dry weight (1,000 kg/ha) of the birch stock								
Age (yrs.)	Leaves	Leaf area ha/ha	No. of leaves ($\times 10^6$)	Living branches	Dead branches	Boles	Whole living shoot	Roots > 0.5 cm diam.
[illegible]	0.1	0.4	4	0.4	0.1	0.9	1.4	0.3
24	2.4	6.2	57	11.8	2.7	48.0	62.2	16.9
27	1.3	3.5	38	8.8	0.7	68.2	78.3	—
32	1.6	4.1	56	9.4	2.8	52.8	63.8	—
[illegible]	0.7	1.7	29	10.9	3.6	58.9	70.5	—
42	1.1	2.8	34	9.1	4.5	58.5	68.7	25.8
46	1.6	4.1	51	16.9	2.0	102.3	120.8	—
53	2.3	5.9	69	30.6	2.5	139.8	172.7	—
57	2.5	6.5	67	27.0	1.7	134.5	164.0	49.8

sharply with comparable results obtained from even-aged plantations of Scots pine in which the weight of branches does not increase greatly after about 20 years of age (Ovington [5]). This fundamental difference between birch and pine woodlands probably reflects differences in the branching habits of the two species, the pine branches tending to be relatively short lived and not continuing to enlarge indefinitely.

The weight of leaves, unlike that of boles or branches, does not continue to increase with age in the eight older stands of birch. Due to the large number of seedlings there is a rapid build up of leaf mass so that at 24 years of age over 2,000 kg of leaf material is present per hectare of woodland. The weight of leaf carried on the eight older stands varies between 700 and 2,500 kg per ha tending to be at a minimum at about 38 years of age.

Jokela and Ylänen [1] working in Finnish birch forests have recorded comparable weights of leaf material and it is interesting that their results also show a temporary decline in leaf weight after about 25 years of age.

Too few roots were sampled to provide comprehensive data of the build up of the root systems in the various plots but it seems clear that the total weight of roots per plot increases with age. The presence of root grafts between neighbouring trees suggests that, when a tree dies, some of its roots may survive and become an effective part of the root systems of other trees. Laitakari [3] considered that the roots of woodland trees may not function individually and Kuntz and Riker [2] by using radioactive fungus

spores and iodine 131, have shown that root grafts permit the transfer of substances from one root to another.

2. *Gross production*

In order to estimate the total production of dry matter (exclusive of roots) by the birch stands, the cumulative losses from the living trees and by trees that have died were added to the weight of the stock (Table VI). After the first few years, when the annual increment of dry matter is low, there is a period of more rapid growth. During this period, the current annual increment averages approx. 7,900 kg per ha. The relative constancy of annual dry-matter production after the birch woodlands close canopy suggests that they are making their fullest use of the site. The dry-matter pro-

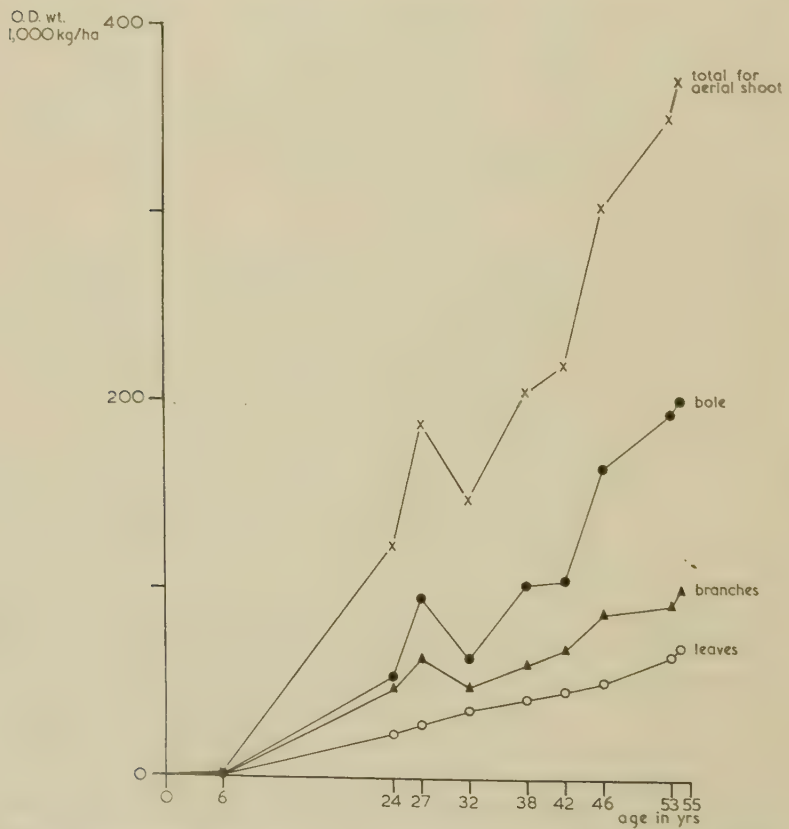

Fig. 3. The gross production of O.D. matter by natural stands of birch.

TABLE VI

The total weight of dry matter (1,000 kg/ha) produced by natural stands of birch (inclusive of dead trees and canopy loss)

After	Branches	Leaves	Bole	Total shoot
6 yrs.	0.6	0.4	0.9	1.9
24 yrs.	46.5	22.8	53.6	122.9
27 yrs.	63.0	28.4	95.2	186.6
32 yrs.	48.3	35.6	62.9	146.9
38 yrs.	60.2	42.3	102.1	204.6
42 yrs.	68.2	45.7	104.1	218.0
46 yrs.	86.9	50.9	164.4	302.2
53 yrs.	91.6	64.2	192.0	347.8
55 yrs.	99.9	69.0	198.7	367.6

duction is made up of about 1.4, 1.9 and 4.6 thousand kg of leaf, branch and bole material respectively.

The total area of leaf per stand varies during the period of rapid growth and relatively constant increment, so that there is some variation in the general efficiency of the leaves for assimilation. The range of values will be affected by experimental errors but for the sampling periods ranged between 0.06 and 0.61 with an average of 0.24 kg of dry matter produced annually by each sq.m of leaf surface (including both sides of the leaves in the area estimates). Birch is deciduous and, if we assume that the leaves are carried for six months only, the average weekly production of dry matter amounts to 9 g/m^2 of leaf area.

DISCUSSION

The stocking density of natural woodlands may greatly exceed that of forest plantations, if there is an adequate supply of viable seed and site conditions are suitable. At six years of age, the number of seedlings at Holme Fen is almost double the number of plants that would be used in normal afforestation planting. Although the natural seedlings are not evenly spaced, the number of seedlings is so great that a close, dense canopy soon develops and forest conditions are established. Consequently, natural stands tend to attain maximum site utilisation for dry-matter production more rapidly than forest plantations.

The current annual increment of dry matter (exclusive of roots) by the birch stands reaches a more or less constant maximum value of about 8,000 kg per ha from twenty years of age onwards. This is

much smaller than the maximum value of 19,000 kg per ha/annum recorded for plantations of *Pinus sylvestris* (Ovington [5]) and for other conifers (Ovington and Pearsall [4]) but is within the general productivity range for different herbaceous types (Pearsall and Gorham [6]) and for agricultural crops in Britain. The difference in productivity between birch and conifer stands does not appear to result from differences in the photosynthetic efficiency of leaves (Watson [8]) or of site conditions but seems to be related to the ability of coniferous crops to carry a larger weight of leaves throughout the whole year. Thus, the plantations of Scots pine not only have a greater leaf area than the stands of birch but are evergreen so that photosynthesis can occur whenever weather conditions are favourable and the annual dry-matter production of the pines is almost twice that of the birch. It is interesting that leaves represent a much smaller proportion of the total dry-matter production in the birch stands (19 per cent) than in plantations of Scots pine (32 per cent).

SUMMARY

At Holme Fen Nature Reserve, two sample trees were felled in each of nine, natural birch stands representing an age series of from 6 to 55 years. Data are given of the weights of leaves, branches, boles and in some cases of roots for the sample trees and the growing stock. Estimates of the gross production of dry matter by birch are given after allowing for the death of suppressed trees and leaf fall *etc.* Attention is drawn to the fundamental differences between the productive capacities of natural birch stands and plantations of evergreen conifers such as *Pinus sylvestris*.

Received April 24, 1958

REFERENCES

1 Jokela, A. and Ylänen, J., Amount of foliage in birch stands. Metsät Aikak. **4**, 131 (1956).

2 Kuntz, J. E. and Riker, A. J., The use of radio-active isotopes to ascertain the role of root grafting in the translocation of water, nutrients, and disease-inducing organisms among forest trees. Proc. Intern. Conf. Peaceful Uses Atomic Energy Geneva 1955 **12**, 144 (1956).

3 Laitakari, E., Männyn juuristo. Morfologinen tutkimus. Acta Forest Fenniae **33**, 1 (1929).

4 Ovington, J. D., and Pearsall, W. H., Production Ecology. 2. Estimates of average production by trees. Oikos **7**, 202 (1956).

5 Ovington, J. D., Dry-matter production by *Pinus sylvestris* L. Ann. Botany N.S. **21**, 287 (1957).
6 Pearsall, W. H. and Gorham, E., Production Ecology. 1. Standing crops of natural vegetation. Oikos **7**, 193 (1956).
7 Rutter, A. J., The relation between dry weight increase and linear measures of growth in young conifers. Forestry **28**, 125 (1955).
8 Watson, D. J., The physiological basis of variation in yield. Advances in Agron. **4**, 101 (1952).

Plant and Soil X, no. 3 March 1959

SOME APPROACHES TO EXPERIMENTAL INVESTIGATIONS INTO THE CORRELATION BETWEEN THE SLOPE AND THE SIGMOIDAL SHAPE OF YIELD CURVES

by F. STEENBJERG and Sv. T. JAKOBSEN

Department of Soil Fertility and Plant Nutrition
The Royal Veterinary and Agricultural College, Copenhagen, Denmark

The yield of a crop and the chemical composition of the dry matter produced are influenced by growth conditions such as soil, climate, and supply of nutrients in the widest meaning of the words.

The principal relationship between supply of nutrients and the crop yield may be expressed graphically when the units on the abscissa represent amounts of nutrient added and on the ordinate the yield of dry matter. Other relationships which may be of interest are derived from or related to this curve, which is generally called the yield curve. Investigations of this kind are often extended to include designs necessitating the use of co-ordinate systems with more than two axes.

In what follows the rising part of the yield curve is treated, *i.e.*, increased yield by an increased supply to the soil of a nutrient ion which is deficient at the beginning of the growing season.

In experimental investigations into the general shape of yield curves the experimental design must be in accordance with the knowledge available from related sciences. Careful analyses of experimental results determined with very small experimental errors have shown that the yield curve is sigmoid when water-soluble nutrients are added to the soil. In a previous paper [9] a theory of the general shape of the yield curve was advanced on an experimental basis.

Much interest has centred on mathematical expressions for the

smoothing of yield curves illustrating experimental results [8]. Suitable equations may be useful in a variety of ways. But a fundamental knowledge of the growth factors, which to a marked degree influence the slope and position of yield curves, and the constants of yield equations can only be obtained by experimental investigations.

Which growth factors are primarily involved and to what extent do we possess a knowledge of the factors which influence the shape of the yield curve? In our experiments the slope observed seems to be correlated with the position of the curve in the system of co-ordinates, the slope and position depending on growth factors, *i.e.*, factors of place and time. However, it must be admitted that a basic knowledge of such correlations is still lacking.

The results obtained so far seem to show that correlations between the slope and the position of the yield curve are influenced by

1) the fixing power of the soil in question for the nutrients concerned,
2) applications of other nutrients not deficient, basic treatment,
3) the available water,
4) the time factor, and
5) the crop [7].

In what follows some general points of view or working hypotheses are elucidated by examples chosen from characteristic experimental results. In this schematic and qualitative discussion of the problem the fact is not fully considered that the different nutrient ions (anions and cations) are fixed with different force and in a different way both in the same soil and in different types of soil.

The experiments were made in pots or in the field; generally the experimental error was comparatively small. These results and the approaches they suggest should prove useful in the designing of further experiments intended to provide a basic knowledge of the possible correlations between the slope and the position of the yield curve in systems of co-ordinates.

RESULTS AND DISCUSSIONS

I. *The fixing power of the soil*

Increased amounts of a nutrient ion — anion or cation — which is

deficient and which is strongly adsorbed, are added to the soil. It is assumed that the nutrient ion is fixed (precipitated) or adsorbed by the soil in accordance with Equation (1) or a similar term.

$$v = \frac{q.z}{d + z} \tag{1}$$

This equation is formally identical with Langmuir's adsorption equation. v is the amount of nutrient ion adsorbed, while z is the equilibrium concentration for the nutrient ion. q and d are constants; q is the maximum adsorption, while d is an expression of the slope. If, *e.g.*, phosphorus is used in the form of superphosphate and if v and z and the added amount of phosphorus x are expressed in the same units, we obtain

$$x = v + z \tag{2}$$

and consequently

$$z = x - v \tag{3}$$

In accordance with Equation (1) the adsorption will be relatively greatest with small additions of phosphorus to the soil.

Let us suppose that the yield curve for phosphorus in the form of superphosphate is measured in pure quartz sand without power of adsorption (normal curve A in Fig. 1), *i.e.*, the x-values are not changed into z-values. The same experiment is then carried out in soil with a great power of adsorption to phosphorus in the form of superphosphate (yield curve B in Fig. 1).

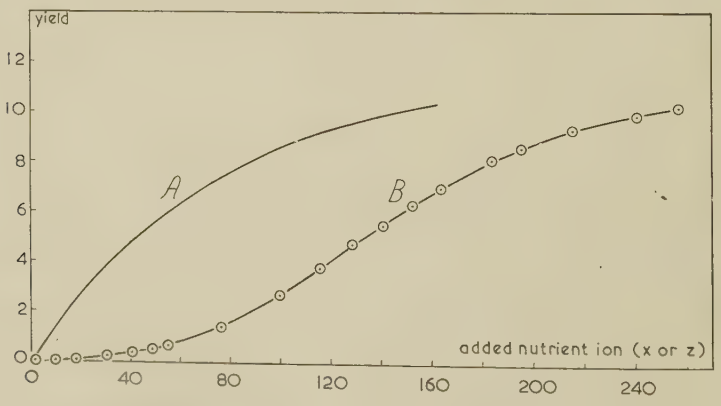

Fig. 1. Schematic presentation of the effect of the fixing power of the soil on the shape of the yield curve. *A* normal curve, *B* computed curve (the computed points designated by circles).

The z-values corresponding to the yield values in Curve B are calculated from the following equation where the constants were chosen to illustrate the point in question

$$v = \frac{100 \cdot z}{5 + z} \tag{4}$$

and from Equation (3).

It is seen from Fig. 1 that the appearance of a sigmoid yield curve can be explained solely by the adsorption by the soil of the nutrient ion which is deficient and which is added to the soil in increasing amounts (see amongst others Gericke [6]).

Fig. 2 shows yield curves for phosphorus. The sigmoid shape can, in the main, be assumed to have been caused by adsorption.

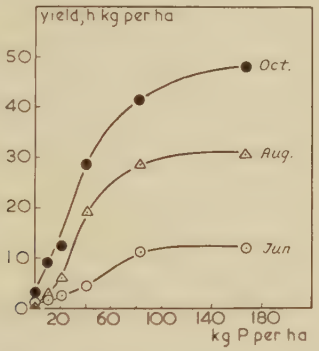

Fig. 2. The relationship between phosphorus added in the form of superphosphate and yield. Pasture. (Rossiter and Ozanne [5]).

The data originate from a paper by Rossiter and Ozanne [5] in Australia. The soil was described as a gravelly sand of lateritic origin. The sigmoid shape of the curves was very pronounced in 1953. Superphosphate was added in 1952. Similar yield curves may be obtained with soils of highly ochreous character or of basaltic origin, where we must assume that there is a marked adsorption of phosphorus especially if the soils are highly deficient in phosphorus. Examples of similar sigmoid yield curves for phosphorus occur in cases of heavy acid clayey soils. Manganese, copper, molybdenum, and various other micronutrients, which are usually strongly adsorbed in deficient soils, most commonly give curves corresponding to the curves in Fig. 2 (see Figs. 5 and 6).

The yield curve in Fig. 3 is an example of a slightly sigmoid curve. Phosphorus was added in the form of superphosphate. The soil was a newly cultivated heath soil poor in nutrients from Skarrild in Jutland, consisting mainly of quartz sand with a small humus content. In both cases (Fig. 2 and Fig. 3) the soils were naturally very deficient in nutrients. It is seen that the curve in Fig. 3 is not by far so markedly sigmoid as the yield curves measured in the lateritic soils in Australia. This may be due to the fact that the *d*-value (Equation (1)) is higher for the soil from Skarrild than for the soil of lateritic origin (Fig. 2).

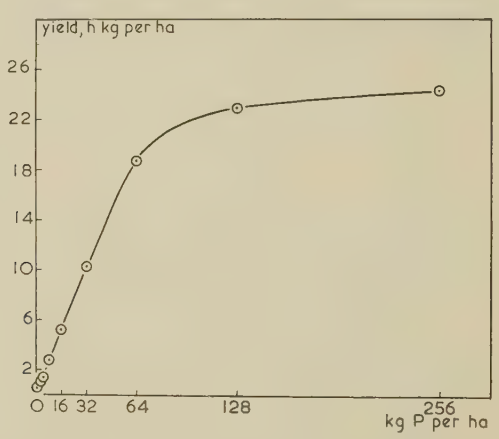

Fig. 3. The relationship between phosphorus added in the form of superphosphate and yield. Oats: grain. (Steenbjerg and Jakobsen [11]).

II. *The nutrients in the basic treatment*

Investigations into the influence of the basal dressing on the slope and position of the yield curve can – for the illustation of the effect of basal dressing – be made with an indifferent growth medium, *e.g.* quartz sand, so that neither the nutrient ion that is deficient and is added in increasing amounts, nor the nutrient ions from the basal dressing which are added in considerable, but constant amounts, are adsorbed by the growth medium.

Actually the nutrients added in the basal dressing will either hardly be adsorbed, or, if adsorbed, they will, as they are added to the soil in a constant amount at the beginning of the experiment, be adsorbed almost to the same extent in all experimental

treatments, and as a rule comparatively less comprehensively in accordance with the shape of the ordinary adsorption isotherms when compared with the smallest amounts added of the nutrient ion that is deficient.

With a heavy basal dressing we may generally expect blocking of the root absorption – caused by the ions in the basal dressing – for the smallest amounts added of the nutrient ion that is deficient *. The mechanism of the blocking is unknown, but the blocking must be supposed to have the comparatively greatest effect at the smallest supplies of the nutrient that is deficient. If the blocking function is similar to that indicated in Equation (1), it means that the blocking is comparatively greatest at the smallest concentrations of the plant nutrient that is deficient. The slope and position of the yield curve must therefore be influenced in the same way with marked blocking – *i.e.* by a heavy basal dressing – as with the adsorption of the nutrient ion that is deficient.

As an example an extract of Mitscherlich, v. Boguslawski, and Gutmann's experimental results will be used [3].

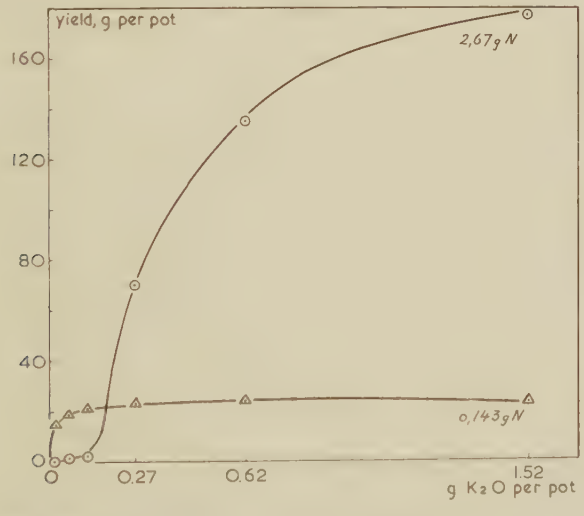

Fig. 4. The relationship between added potash and yield. Oats: grain + + straw. (Mitscherlich, v. Boguslawski und Gutmann [3]).

* A varying blocking of the root absorption (at the concentrations of equilibrium) for the nutrient ion that is deficient may be caused by a varying competition of absorption at the root surface of the nutrient ions from the basal dressing.

The experiments were carried out in pots. The growth medium was tertiary quartz sand from Hohenbocka near Dresden. 1.5 g P_2O_5 as primary sodium phosphate, 1 g magnesium sulphate, 0.5 g sodium chloride, and 1.5 g calcium carbonate were added as a basal dressing. The supply of nitrogen was varied in the form of ammonium nitrate. The effect of two different amounts of nitrogen is shown in Fig. 4. The supplies of potassium were varied in the form of potassium sulphate. The heaviest dressing with ammonium nitrate blocks the absorption of potassium at the smallest level of potassium sulphate. The blocking is relatively decreased with increasing supplies of potassium sulphate (see also Hagin [1]). In accordance with this view of blocking (pronounced plasmolysis may perhaps be contributory in the case of a heavier basal dressing) we should, when using normal, sandy soils as a growing medium be able to find by careful measuring sigmoid yield curves even for nitrate ion (Poulsen [4]).

In future experimental work it may be possible to change the shape of the yield curve by combining hypotheses I and II. If, *e.g.*, the nutrient ion that is deficient shows high q- and low d-values (Equation (1)) in the growth medium in question, and a heavy basal dressing with other nutrients which are not particularly firmly fixed is added to the soil, the yield curve should follow a steep slope and reach a high position in the co-ordinate system. It should be more markedly concave in the first part of the curve than the curve for a growth medium which shows, *e.g.*, the same q-value, but a considerably higher d-value for the nutrient ion that is deficient or to which soil has been added a light basal dressing or which has a high power of adsorption for the nutrient ions added with the basal dressing. It is presupposed that the blocking is dependent on the equilibrium concentrations of both the deficient nutrient and the basal dressing.

III. *The available water*

The amount of water available to the plants during the growing season depends on the amount of rainfall, irrigation, and the soil type. With this schematic treatment we disregard variations in rainfall distribution within the growing season.

Apart from nitrate ion, which is not notably fixed in ordinary arable soil, the nutrient ions are adsorbed to a varying extent,

especially dependent on the kind of the nutrient ion and the soil and, furthermore, on the amounts and combinations of the ions present.

The following general question may be asked: in what way does the content of water in the soil influence the equilibrium concentration of a) the nutrient ions supplied to the soil in constant amounts (the basal dressing), and b) the nutrient ion that is deficient?

a) In accordance with the general shape of the adsorption isotherm the nutrient ions of the basal dressing are fixed relatively less comprehensively in dry years, and their equilibrium concentration in the soil is higher than in a year of high rainfall. The blocking – at the lowest concentrations of the nutrient ion that is deficient – is thus most comprehensive in dry years. Hence there would be a possibility of observing a pronounced concavity in the first part of the curve during dry conditions.

b) On the other hand, the adsorption and the equilibrium concentration of the nutrient ion that is deficient must also be influenced by a different soil-water content. The adsorption will be relatively smallest in dry years and the equilibrium concentrations highest, which may reduce the concavity in the first part of the curve.

The relative importance of the two possibilities – a predominant effect of an increased blocking in dry years of the nutrient ion that is deficient or an increased equilibrium concentration in dry years of the nutrient ion that is deficient – may be tested by experiments. The final result must, in general, depend on the type of nutrient ion, the type of soil, and of the kind, amount, and composition of the basal dressing, *i.e.* on the q- and d-values for adsorption and the q_1- and d_1-values for blocking.

The yield curves [10] for copper in Figs. 5 and 6 may be interpreted in the following way: the yield curves measured in the year 1942, a year of heavy rainfall, have a steeper slope in their middle part than those found for the year with low rainfall, and they also have a pronounced concavity in their first (lower) course. The latter fact is observed best in Fig. 6, where the extrapolated curves are seen in their entirity. It should be mentioned that the absorption quotient for copper was highest in the wet year. This suggests that a) the change in the conditions of blocking with the two

different supplies of water causing different equilibrium concentrations for the nutrient in the basal dressing was of less importance for the shape of the curves than b) the influence of the different conditions of humidity on the adsorption of the smallest amounts of copper.

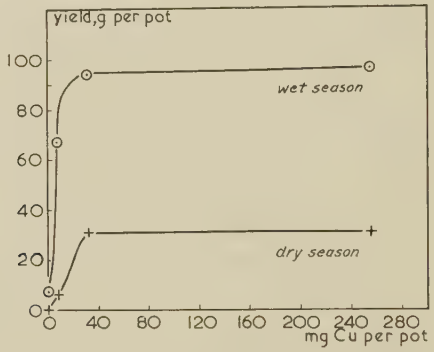

Fig. 5. The relationship between added copper and yield. Barley, grain. (Steenbjerg and Boken [10]).

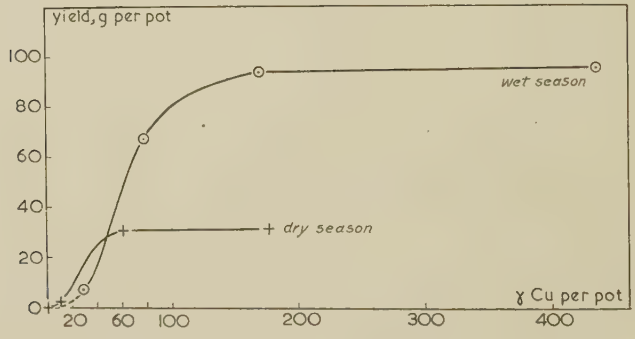

Fig. 6. The relationship between absorbed copper and yield. Barley, grain. (Steenbjerg and Boken [10]).

Observations of the yield curves of phosphorus (phosphorus added to the soil in the form of superphosphate) determined in soil with a high *d*-value for phosphorus as compared with copper suggest that blocking by the basal dressing may be a dominant factor under dry conditions, so that the concavity is most pronounced under dry conditions: Tarm Østerhede, ploughed to a depth of 75 cm represented in 1957 a dryer soil than when ploughed

to 45 cm (see Fig. 7). It should, however, be mentioned that the ferric-ion content of the surface-soil layer seemed to be highest with a 75-cm ploughing which brought gravelly yellow and brown sand from the B_1-, B_2-, and C-horizon to the toplayer of soil. The two experimental sites were placed side by side.

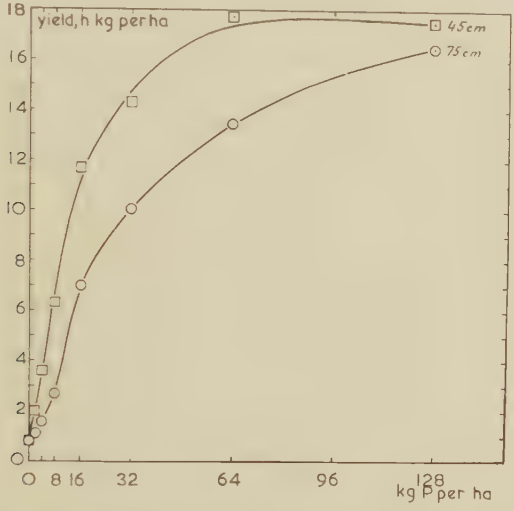

Fig. 7. The relationship between phosphorus added in the form of superphosphate and yield. Oats (grain + straw). (Steenbjerg and Jakobsen [11]).

It appears from Fig. 6 that the percentage content of copper falls by increasing absorption of copper, reaches a minimum, and then increases again by further absorption of copper (see also Ref. [8]).

IV. *The time factor*

The importance of the time factor for the correlation between the slope and the position of the curve is insufficiently elucidated. Investigations made at this institute show that with a nutrient ion that is deficient and is added to the soil in increasing amounts, the above-mentioned variation in the percentage contents of the nutrient ion in question and the corresponding utilization quotients (reciprocal percentage contents) may be greatest at the beginning of the growing season.

The extent of the adsorption of nutrient ions in the soil is de-

pendent on time. The graph of the time function for the available content of nutrients in the soil is as a rule a falling concave curve. This means that the blocking of a nutrient ion that is deficient and is adsorbed relatively quickly and comprehensively by the soil, must be most pronounced at the beginning of the growing season, when the nutrient ions in the basal dressing added is least comprehensively adsorbed. This may be the reason why the trend in the percentage content of nutrients and in the utilization quotients for the nutrient that is deficient (see above), is often most pronounced at the beginning of the growing season, especially for the lowest part of the curve, *i.e.*, as far as the tangent point which is found by drawing a straight line from the point of intersection of the axes of the system of co-ordiantes in Fig. 6 (see Ref. [8]).

For nutrient ions such as copper and manganese a quick and comprehensive adsorption in the soil may be observed in soils deficient in these two elements. The extent of the adsorption of the nutrient ions from the basal dressing, *e.g.* phosphorus and potassium, is generally increased for a longer period in such soils. The different extent of the adsorption together with the different dependence on time of the adsorption with these two groups of nutrient ions – manganese and copper, and the nutrient ions in the basal dressing – may be a contributory explanation of the great variations in the percentage contents of manganese and copper and the corresponding utilization quotients, which have been observed at the beginning of the growing season [2].

SUMMARY

On the basis of selected experimental data a preliminary working hypothesis is advanced which may be the starting-point of experimental work for the establishment of the correlation between the slope of the sigmoid yield curve and its position in the system of co-ordinates. The hypothesis takes into account the fixing power of the soil for the deficient nutrients and for the nutrients in the basal treatment as influenced by nutrient concentrations and by the water content of the soil and the time factor.

Received July 8, 1958

LITERATURE CITED

1 Hagin, J., A study on the shape of the yield curve. To be published.

2 Jacobsen, Ingeborg, Om planternes manganindhold som hjaelpemiddel ved bestemmelse af manganmangel. (On the manganese contents of crops as a diagnostic measure of manganese deficiency). Horticultura nr. 10, p. 89–97 (1951).

3 Mitscherlich, E. A., Boguslawski, E. von, and Gutmann, A., Studien über die Ernährung der Pflanze und die Ertragsbildung bei verschiedener Düngung. Schriften Königsberg. Gelehrten Ges. Naturw. Kl. **12**, 31–117 (1936).

4 Poulsen, E., Stofproduktion og næringsoptagelse hos sukkerroer med særligt henblik på udbyttekurvernes funktion ved store gødningstilførsler. Hovedopgave ved specialkursus i landbrugets jorddyrkning, p. 1–47 (1956).

5 Rossiter, R. C. and Ozanne, P. G., The shortterm effects of rock phosphate and superphosphate on a subterranean clover pasture. Australian J. Agr. Research **6**, 553–564 (1955).

6 Scheffer, Fr. and Welte, E., Lehrbuch der Agrikulturchemie und Bodenkunde. II. Teil, Pflanzenernährung. Stuttgart. 1955. Gericke. *loc. cit.* p. 219. Abh. 46. (1955).

7 Steenbjerg, F., On the relative contents of plant nutrients in crops. Trans. Intern. Congr. Soil Sci. 4th Congr. Amsterdam **I**, 254–258 (1950).

8 Steenbjerg, F., Manuring, plant production and the chemical composition of the plant. Plant and Soil **V**, 226–242 (1954).

9 Steenbjerg, F., A theory on the placement of fertilizers. Roy. Vet. and Agr. Coll. Copenhagen Den. Yearbook **1957**, 1–30 (1957).

10 Steenbjerg, F. and Boken, Else, Kobber i Jord og Kulturplanter III. Kobberindhold og Kobbermangel i jydske Jordtyper. (Copper in Soils and Cultivated Plants III. Copper contents and copper deficiency in Jutlandish soil types). Tidsskr. Planteavl **52**, 375–459 (1949).

11 Steenbjerg, F. and Jakobsen, Sv. T., To be published.

LETTER TO THE EDITORS

Modifying Effect of EDTA on the Copper Toxicity to Corn in Nutrient Solution

Chelated iron has been successfully used as a source of iron in nutriculture. The authors observed in a previous publication [3] that EDTA in low concentrations (10 micromoles or below) is beneficial to the growth of corn (maize) in solution culture. Because of the low molar ratio of EDTA to Fe^{++} ions, this beneficial effect could not have been due to better availability of the iron. High concentrations of Cu or Mn in the growth medium have been suggested as a cause of iron chlorosis and that a chelating agent may correct this condition. This experiment was designed to gain information on the modifying effect of EDTA on copper toxicity to corn in nutriculture.

The growing conditions for the cultures and the make-up of the basic nutrient solution was the same as described previously [4]. There were two sets of 6 treatments – each treatment consisting of 5 culture jars. The treatments of one set received 6 different concentrations of $CuSO_4$, *e.g.*, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 micromoles. The other set was identical with the first one except that 5 micromoles of the disodium salt of EDTA were added to each culture jar of all the treatments. The pH of the solutions remained between 6.0 and 7.0 throughout the experimental period. Results are shown in Table I.

It is evident from these results that EDTA can modify copper toxicity in corn to a considerable extent. Plants receiving 0.1 and 0.2 micromoles of $CuSO_4$ did not show any visible abnormality in growth regardless of the presence or absence of EDTA in the solution. With increasing concentrations of copper the growth of plants receiving no EDTA became progressively poorer and was accompanied by severe chlorosis and browning of roots. These are known to be symptoms of copper toxicity. Plants receiving EDTA, however, showed no signs of copper toxicity, although there was a trend of depression of growth with increasing amounts of copper. This was far less drastic than in cultures without EDTA (Table I). Furthermore, the dry-weight yields of most of the treatments with EDTA were significantly higher than those of their counterparts without this substance. Root growth, especially, was promoted considerably by the chelate, even at high concentrations of copper.

The results are in substantial agreement with those of other workers. Chelated iron has been observed to reduce heavy-metal toxicity in nutrient

TABLE I

Modifying effect of EDTA on the copper toxicity in corn grown in solution culture

Treatments	Mean dry weight g per plant		Standard error	
	Top	Root	Top	Root
× 1 Cu	2.09	1.16	0.133	0.081
× 2 Cu	2.11	0.98	0.088	0.073
× 4 Cu	1.35	0.80	0.157	0.057
× 6 Cu	0.66	0.42	0.093	0.052
× 8 Cu	0.48	0.38	0.025	0.031
× 10 Cu	0.43	0.36	0.039	0.018
× 1 Cu and EDTA	2.40	1.24	0.229	0.090
× 2 Cu and EDTA	2.73	1.35 *	0.232	0.073
× 4 Cu and EDTA	2.97 **	1.44 **	0.118	0.073
× 6 Cu and EDTA	2.26 **	1.09 **	0.143	0.087
× 8 Cu and EDTA	2.24 **	1.08 **	0.151	0.064
× 10 Cu and EDTA	1.64 **	0.84 **	0.140	0.059

The designations × 1 Cu, × 2 Cu, × 4 Cu, × 6 Cu, × 8 Cu, and × 10 Cu indicate 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 micromoles of $CuSO_4$ per liter respectively.

* Significantly higher (at 0.05 level) than the value in the corresponding treatment receiving no EDTA.

** Significantly higher (at 0.01 level) than the value in the corresponding treatment receiving no EDTA.

solutions. The same material, either as a foliar spray or as a soil amendment, has been effective in correcting iron chlorosis even in the presence of high amounts of copper. The improvement of the root systems even at high copper concentrations here observed was somewhat at variance with results obtained elsewhere.

The biochemical basis for this modifying effect of EDTA on copper toxicity in solution culture is open to speculation. Metal ions vary in the stability of the complexes they form with EDTA; and in a scale descending from strong to weak chelates, the order is Fe^{+++}, Cu^{++}, Zn^{++}, Fe^{++}, Mn^{++}, Ca^{++}, and Mg^{++} (see Ref. [2]).

It is known, however, that due to chemical or photochemical reactions the Fe^{+++} ions in the solution become reduced to Fe^{++} form. Then the Cu^{++} ions take priority in any chelating action. The ratio of EDTA to Cu^{++}, even in the treatment receiving the highest concentrations of copper, was considerably higher than 1 : 1. In the event of reduction of ferric ions to ferrous ions, all the cupric ions in all the treatments receiving EDTA should be chelated. Results presented here in Table I indicate, however, that complete chelation of cupric ions, particularly above the level of 0.4 micromoles, is not necessarily a corrective for the copper toxicity and that high concentrations of copper can interfere with the effectiveness of EDTA as a growth-stimulant as observed by the present authors previously [1].

This report is based on portions of a thesis presented by the senior author in partial fulfillment of the requirements for the Ph.D. degree and published with the approval of the director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 227.

S. K. Majumder and Stuart Dunn
Botany Department, University of New Hampshire,
Durham, New Hampshire, U.S.A.

Received July 22, 1958

REFERENCES

1 Majumder, S. K., and Dunn, S., Effects of metal chelates on growth of corn in solution culture. Plant Physiol. **33**, 166–169 (1958).
2 Wallace, A. (ed.), Symposium on the use of metal chelates in plant nutrition. The National Press, Palo Alto, California (1956).

LETTER TO THE EDITORS

The Influence of Certain Micro-nutrient Deficiencies on the Germination Rates of Radish and Millet

The influence of micro-nutrient deficiencies on the germination of seeds does not appear to have been investigated. If the water supply to the seed is adequate, the micro-nutritional status of the environment might not be expected to influence germination appreciably, as the seed has its own reserves of nutrients. However, observations made in this laboratory during other experiments indicated that this was not the case. The micro-nutrient status of the environment does significantly influence germination, and some aspects of this phenomenon were quantitively investigated.

The rates of germination of radish (*Raphanus sativus*) and of millet (*Pennisetum glaucum*) seeds were studied at four temperatures, 15°, 20°, 25° and 30°C. Seeds were surface-sterilized rapidly in 0.2% mercuric chloride. Radish seeds were then soaked for 24 hours in Robbins' solution [1], or in a Robbins' solution lacking B, Cu, Mn or Zn respectively in the case of deficient solutions. Millet, because of its rapid germination, was only soaked for 12 hours. The seeds were then planted 1 cm below the surface of acid-washed sand, at the rate of 20 seeds per glass pot, and 10 pots per treatment. The sand was wetted with the relevant solutions. All conditions of temperature, quantities of sand, solutions *etc.*, were standardised, and treatments were comparable.

TABLE I

Radish seeds germinated in sand.

All seeds grown in deficient solutions germinated more rapidly than the controls. This table shows the high degree of significance in the variation from the control. The significance of the variations from the control were calculated by the Chi-square method.

Dashes indicate no significant difference from controls.

Temperature	Time in hours	Deficient element	Significance level
25°C	48	Cu	1%
		Zn	2%
	57	Cu	1%
	72	Cu	5%
		Mn	5%
	82	Cu	1%
	96	—	—

Germination was measured by the emergence of the seedling above the sand. All the deficient treatments germinated more rapidly than the controls. Table I shows the treatments that were statistically highly significant. In no case, do the various treatments significantly alter the percentage of germinating seeds, but they do alter the rate of germination. In this case, Cu-deficiency greatly enhances the rate.

TABLE II

Degrees of significance in variation from controls in shoot growth rates of seeds germinated on filter paper at different temperatures and with different solutions. All show more rapid germination of seeds in deficient substrates.

Temperature	Time in hours	Deficient element	Significance level
Radish			
15°C	24	—	—
	36	B	5%
		Cu	2%
	48	—	—
	57	Zn	2%
	74	—	—
	82	—	—
20°C	No significant difference in any treatment		
25°C	30	B	5%
	48	—	—
	72	Zn	5%
	57	—	—
30°C	36	Zn	5%
	48	B	1%
		Cu	1%
		Mn	1%
		Zn	1%
	58	B	1%
		Mn	2%
	72	B	1%
		Mn	5%
	84	B	1%
		Mn	5%
	96	B	1%
Millet			
15°C	12	B	1%
	18	—	—
20°C	12	—	—
	15	B	1%
		Cu	1%
		Mn	2%
		Zn	1%
	24	Zn	5%
25°C	12	Cu	2%
	15	—	—
30°C	4	—	—

Germination tests by this sand method are not entirely satisfactory because germination is a complex process consisting of two main phenomena; sprouting, *i.e.* radicle growth, and shoot growth. These two processes can occur at different rates, and either, or both of them, can result in germination as measured by emergence above the sand. To measure these two processes separately, seeds were treated as described above and left to germinate on filter paper soaked in the respective solutions in Petri dishes. Then both sprouting and shoot growth could be measured.

Again it was found that in all deficient treatments shoot growth was more rapid than in the controls. In the case of the radish at 30°C, all treatments differed significantly from the controls (Table II). On the other hand, at 20°C no highly significant difference could be determined.

TABLE III

Degree of significance in the variation from controls in sprouting rates of seeds germinated on filter paper at different temperatures and with different solutions. All show more rapid sprouting than controls.

Temperature	Time in hours	Deficient element	Significance level
Radish			
15°C	48	—	—
	72	—	—
20°C	48	—	—
	72	—	—
25°C	48	—	—
	72	—	—
30°C	48	B	1%
		Cu	1%
		Mn	1%
		Zn	1%
Millet			
15°C	12	B	1%
	18	—	—
20°C	12	—	—
	15	B	1%
		Zn	1%
		Mn	2%
		Cu	2%

TABLE IV

Degree of significance in variations from the control in sprouting rates of radish seeds germinated in sand

Temperature	Time in hours	Deficient element	Significance level
25°C	48	Cu	1%
		Mn	1%
	72	Cu	5%
		Mn	5%

The rate of sprouting is also influenced by the micro-nutrient status of the substrate, the seeds on deficient substrates sprouting more rapidly than the controls, although sprouting does not appear to be so greatly influenced by deficiencies as is shoot growth (Table III). If sprouting is measured by the sand method, significant differences are also obtained but the results do not agree with the filter-paper method (Table IV).

Summary

Investigation into the influence of deficiencies of B, Cu, Mn and Zn in the substrate of germinating radish and millet seeds, shows that these deficiencies significantly increase the rate of sprouting and of shoot growth. It also shows that deficiencies in general appear to have greater stimulating effect at higher than at lower temperatures. Two techniques for germination were used in these investigations, one of which germinated seeds on filter paper, the other germinated them in sand. These methods did not give comparable results.

We wish, gratefully, to acknowledge the kind assistance and advice of Mr. J. M. Darroch in statistical matters.

K. H. Schütte and P. Martin
Botany Department, University of Cape Town, South Africa

Received August 25, 1958

LITERATURE

1 Robbins, W. R., Growing plants in sand cultures for experimental purposes. Soil Sci. **62**, 3 (1946).